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 (54) Title: <b>RECOMBINANT SOLUBLE Fc RECEPTORS</b>			
 (57) Abstract			
<p>Recombinant soluble Fc receptors according to the present invention are characterized by the absence of transmembrane domains, signal peptides and glycosylation. Such Fc receptors can easily be obtained by expressing respective nucleic acids in prokaryotic host cells and renaturation of the obtained inclusion bodies, which procedure leads to a very homogenous and pure product. The products can be used for diagnostic as well as pharmaceutical applications and also for the generation of crystal structure data. Such crystal structure data can be used for the modelling of artificial molecules. A further embodiment comprises coupling the Fc receptors according to the invention to solid materials like chromatography materials that can be used to separate and/or enrich antibodies.</p>			

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**Recombinant soluble Fc receptors****Specification**

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The present invention relates to recombinant soluble Fc receptors (FcR), recombinant nucleic acids coding for such Fc receptors, host cells containing corresponding nucleic acids as well as a process for the determination of the amount of antibodies of a certain type contained in the 10 blood, plasma or serum of a patient, a process for the determination of the immune status of patients with chronic diseases of the immune system and a process for the screening of substances in view of their ability to act as inhibitors of the recognition and binding of antibodies to the respective 15 cellular receptors. Further, the present invention is concerned with pharmaceutical compositions containing the recombinant soluble FcRs, crystalline preparations of FcRs and FcR/Ig-complexes and especially of the use of such crystalline preparation for the generation of crystal structure data of Fc receptors as well as FcR inhibitors and pharmaceutical compositions containing such FcR inhibitors.

20

A still further subject of the present invention is a recombinant Fc receptor coupled to a solid phase, e.g. a chromatography carrier material. The use of such chromatography material, which is another subject of the present invention, lies in the absorption of immunoglobulins from a body fluid of 25 patients or from culture supernatants of immunoglobulin producing cells.

Fc receptors (FcRs) play a key role in defending the human organism against infections. After pathogens have gained access to the blood circulation they are opsonized by immunoglobulins (Igs). The resulting immunocomplexes bind due to their multivalency with high avidity to FcR bearing cells leading 30 to clustering of the FcRs, which triggers several effector functions (Metzger, H., 1992A). These include, depending on the expressed FcR type and

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associated proteins, endocytosis with subsequent neutralization of the pathogens and antigen presentation, antibody-dependent cellular cytotoxicity (ADCC), secretion of mediators or the regulation of antibody production (Fridman et al, 1992; van de Winkel and Capel, 1993).

5

Specific FcRs exist for all Ig classes, the ones for IgG being the most abundant with the widest diversity. Together with the high affinity receptor for IgE (Fc $\epsilon$ RIa), Fc $\gamma$ RI (CD64), Fc $\gamma$ RII (CD32) and Fc $\gamma$ RIIIa (CD16) occur as type I transmembrane proteins or in soluble forms (sFcRs) but also a glycosylphosphatidylinositol anchored form of the Fc $\gamma$ RIII (Fc $\gamma$ RIIIb) exists. Furthermore, Fc $\gamma$ Rs occur in various isoforms (Fc $\gamma$ RIa, b1, b2, c; Fc $\gamma$ RIIa1-2, b1-3, c) and alleles (Fc $\gamma$ RIIa1-HR, -LR; Fc $\gamma$ RIIIb-NA1,-NA2) (van de Winkel and Capel, 1993). In contrast to the overall homologous extracellular parts, the membrane spanning and the cytoplasmic domains differ. They may be deleted entirely or be of a size of 8 kDa. They may contain either a 26 amino acid immunoreceptor tyrosine-based activation motif (ITAM) as in Fc $\gamma$ RIIa or a respective 13 amino acid inhibitory motif (ITIM) in Fc $\gamma$ RIIb involved in signal transduction (Amigorena et al, 1992).

20 Judged by the conserved spacing of cysteins, the extracellular part of the FcRs consists of three (Fc $\gamma$ RI, CD64) or two (Fc $\epsilon$ RI, Fc $\gamma$ RII, CD32 and Fc $\gamma$ RIII, CD16) Ig-like domains (10 kDa/domain) and therefore belongs to the immunoglobulin super family. These highly glycosylated receptors are homologues, and the overall identity in amino acid sequence among the Fc $\gamma$ Rs and Fc $\epsilon$ RIa exceeds 50% in their extracellular regions. Nevertheless, the affinity of FcRs to their ligands varies widely. The higher affinity of  $\approx 10^8 M^{-1}$  of the Fc $\gamma$ RI to Fc-fragment is assigned to its third domain, while the other Fc $\gamma$ Rs with two domains have an affinity to IgG varying between  $10^5$  and  $10^7 M^{-1}$ . The affinity of the two domain Fc $\epsilon$ RIa to IgE exceeds these values by far with a constant of  $10^{10} M^{-1}$  (Metzger, H., 1992B). In contrast to the mentioned FcRs the low affinity receptor for IgE Fc $\epsilon$ RII represents a type transmembrane protein and shows a lower homology.

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- Fc $\gamma$ Rs are expressed in a defined pattern on all immunological active cells. Fc $\gamma$ RI is constitutively expressed on monocytes and macrophages and can be induced on neutrophils and eosinophils. The physiological role of Fc $\gamma$ RI is still unknown as the expression on monocytes is not vital (Ceuppens et al, 1988). The GPI anchored form of Fc $\gamma$ RIII (Fc $\gamma$ RIIIb) is exclusively expressed on granulocytes. Due to its missing cytoplasmic part, the signal transduction into the cell occurs solely via other transmembrane proteins like complement receptor type 3 (CR3) that can at least associate with Fc $\gamma$ RIIIb (Zhou et al, 1993; Poo et al, 1995). Fc $\gamma$ RIIa is mainly expressed on monocytes and macrophages but only in conjunction with associated proteins (e.g.  $\alpha$ - or  $\gamma$ -chains). Fc $\gamma$ RII is the receptor with the widest distribution on immunocompetent cells and is mainly involved in the endocytosis of immunocomplexes.
- 15 Fc $\gamma$ RIIa and Fc $\gamma$ RIIb differ in their extracellular region by only 7% of the amino acid residues. Nevertheless, both forms can be distinguished by their binding characteristics to human and mouse IgG subclasses (van de Winkel and Capel, 1993) and their differing affinity to human IgGs (Sondermann et al, 1998A). The situation is rendered even more complicated by the high 20 responder/low responder (HR/LR) polymorphism of Fc $\gamma$ RIIa named after the ability of T cells from some individuals to respond to murine IgG1-induced mitogenesis (Tax et al, 1983). Later, it was found that the two exchanges in the amino acid sequence between the LR and the HR form modify the ability to bind human IgG2, which leads to the suggestion that at least one 25 of them is involved in IgG binding (Hogarth et al, 1992).

In contrast to the beneficial role FcRs play in the healthy individual, they also transmit the stimulation of the immune system in allergies (Fc $\epsilon$ RIa) or autoimmune diseases. Moreover, some viruses employ Fc $\gamma$ Rs to get access 30 to cells like HIV (Homsy et al, 1989) and Dengue (Littaua et al, 1990) or slow down the immune response by blocking Fc $\gamma$ Rs as in the case of Ebola (Yang et al, 1998) and Measles (Ravanel et al, 1997).

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Hence, the object underlying the present invention was to provide receptors which are easy to produce and can advantageously be used for medical or diagnostic applications. Moreover, it was an object of the invention to provide soluble receptors exhibiting a binding specificity and activity which  
5 is analogous to that of the receptors occurring naturally in the human body and which, additionally, make it possible to produce crystals suitable for a structure determination.

This object is accomplished by recombinant soluble Fc receptors which  
10 consist only of the extracellular portion of the receptor and are not glycosylated. The receptors according to the present invention are therefore characterized by the absence of transmembrane domains, signal peptides and glycosylation.

15 Particularly preferred for the present invention are Fc $\gamma$  or Fc $\epsilon$  receptors. This is because IgG and IgE molecules are characteristic for a multiplicity of diseases and conditions, so that their determination and possible ways of influencing them are of great interest. Figure 11 and 12 show an alignment of amino acid sequences of the extracellular parts of some Fc $\gamma$ Rs and Fc $\epsilon$ RI.  
20 The FcRs according to the invention include all these sequences or parts thereof that still retain binding capacity to antibodies and/or proper crystallization.

In a particularly preferred embodiment of the invention the recombinant  
25 soluble FcR is a Fc $\gamma$ RIIb receptor. Further, it is particularly preferred that the receptor be of human origin. In a particularly preferred embodiment, it contains an amino acid sequence as shown in one of SEQ ID NO:1 to SEQ ID NO:6.

30 According to the present invention, the preparation of the soluble Fc receptors preferably takes place in prokaryotic cells. After such expression, insoluble inclusion bodies containing the recombinant protein form in

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prokaryotic cells, thus facilitating purification by separation of the inclusion bodies from other cell components before renaturation of the proteins contained therein takes place. The renaturation of the FcRs according to the present invention which are contained in the inclusion bodies can principally 5 take place according to known methods. The advantage of the preparation in prokaryotic cells, the production of inclusion bodies and the thus obtained recombinant soluble Fc receptors make it possible to obtain a very pure and, in particular, also very homogeneous FcR preparation. Also because of the absence of glycosylation the obtained product is of great 10 homogeneity.

Soluble Fc receptors hitherto produced by recombinant means particularly exhibited the disadvantage that a much more elaborate purification was required, since they were expressed in eukaryotic cells and, due to the 15 glycosylation which is not always uniform in eukaryotic cells, these products were also less homogeneous.

The recombinant soluble Fc receptors according to the present invention even make it possible to produce crystals suitable for use in X-ray analysis, 20 as shall be explained lateron in the description of further embodiments of the invention. The FcRs of the present invention moreover exhibit practically the same activity and specificity as the receptors naturally occurring in vivo.

A further subject matter of the present invention is a recombinant nucleic 25 acid having a sequence coding for a recombinant soluble Fc receptor according to the present invention.

The nucleic acid according to the present invention may contain only the coding sequences or, additionally, vector sequences and/or, in particular, 30 expression control sequences operatively linked to the sequence encoding the recombinant FcR, like promoters, operators and the like.

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In a particularly preferred embodiment the nucleic acid of the present invention contains a sequence as shown in one of SEQ ID NO:7 to SEQ ID NO:12. For a comparison, SEQ ID NO:13 and SEQ ID NO:14 show the respective wild type sequences coding for Fc $\gamma$ RIIb and Fc $\epsilon$ Rla. SEQ ID NOs:15-18 show the wild type sequences for Fc $\gamma$ RI, Fc $\gamma$ RIIa, Fc $\gamma$ RIII and Fc $\epsilon$ RII.

If the nucleic acid of the present invention contains vector sequences, then these are preferably sequences of one or several prokaryotic expression vectors, preferably of pET vectors. Any other known functions or components of expression vectors may also be contained in the recombinant nucleic acid according to the present invention if desired. These may, for instance, be resistance genes allowing for an effective selection of transformed host cells.

15

A still further subject matter of the present invention is a host cell containing a recombinant nucleic acid according to the present invention. As repeatedly mentioned above, the host cell preferably is a prokaryotic host cell, particularly an E. coli cell.

20

The recombinant soluble Fc receptors according to the present invention can be used for a multitude of examinations or applications because they specifically react with antibodies. In vivo, the soluble Fc receptors are powerful immunoregulators which, if present in elevated levels, result in a remarkable suppression of the immune system which leads to many partly known and partly not yet understood effects. Based on these effects, several applications of the Fc receptors according to the present invention are further subject matters of the present invention.

30

One such subject is a process for the determination of the amount of antibodies of a certain type in the blood or serum of a patient, which is characterized by the use of a recombinant soluble FcR according to the

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invention in an immunoassay, and the determination of the presence of FcR-antibody complexes. Such assay allows to screen for the presence of a certain kind of antibody and allows also for the determination of the amount of antibodies present in the blood, plasma or serum of a patient.

5

Any type of immunoassay is principally suitable for the use according to the present invention, as long as the presence of FcR-antibody complexes can thereby be detected. Both ELISA (enzyme-linked immunosorbent immunoassay), particularly sandwich assays, and RIA (radio-immunoassay) 10 are suitable, but also competitive testing methods. In a preferred embodiment of the invention where the presence and/or the amount of IgE antibodies is to be examined, an Fc $\epsilon$ R is used as recombinant soluble receptor according to the present invention. In particular, this method is suited and advantageous for determining a predisposition or manifestation 15 of an allergy.

Moreover, a method is preferred in which the presence of soluble FcRs is to be determined and, if required, quantified. For such determination preferably a competitive immunoassay method is used, wherein as competition reagent a recombinant soluble receptor according to the invention is used, most preferably a recombinant Fc $\gamma$ R. By means of this test among others the immune status of patients with chronic diseases of the immune system can be determined in a competitive immunoassay. Chronic diseases in the sense of these processes are for instance AIDS, SLE 20 (systemic lupus erythematosus), MM (multiple myeloma) or rheumatoid arthritis, or in the case of Fc $\epsilon$ RII in B-CLL (Gordon et al., 1987), hyper IgE syndrome (Sarfati et al., 1988) or HCL (Small et al., 1990).

A further advantageous use of the recombinant receptor according to the 30 present invention lies in the screening of substances in view of their ability to act as inhibitors of the recognition and binding of antibodies to the respective cellular receptors.

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By means of modern screening techniques such as HTPS (high throughput screening) in combination with multi-well microtiter plates and automatic pipetting apparatuses it is nowadays possible to simultaneously test a multitude of substances for specific properties. As the FcRs according to 5 the present invention can be easily produced at low cost, they can also be used in such series tests by which substances having an inhibiting effect can easily be identified.

Particularly preferred is such use according to which Fc receptors according 10 to the present invention are used to find or screen inhibitors capable of inhibiting the recognition and binding of the respective antibodies to the particular receptor of interest.

A further area of application of the substances according to the invention 15 lies in the pharmaceutical field. Hence, a further subject matter of the invention is a pharmaceutical composition comprising as active agent a recombinant soluble FcR according to the invention. According to the present invention, this pharmaceutical composition may of course comprise conventional useful carrier and auxiliary substances. Such substances are 20 known to the person of skill in the art, the mode of administration also having to be taken into account. The pharmaceutical composition of the present invention can be advantageously used for the treatment or prevention of autoimmune diseases, allergies or tumor diseases.

25 Soluble forms of Fc receptors such as FcγRIII mediate isotype-specific regulation of B cell growth and immunoglobulin production. In a murine model of myeloma, sFcR suppresses growth and immunoglobulin production of tumor cells (Müller et al, 1985; Roman et al, 1988; Teillaud et al, 1990). Furthermore, sFcR binds to surface IgG on cultures of human IgG-secreting 30 myeloma cells and effects suppression of tumor cell growth and IgG secretion. Prolonged exposure of these cells to sFcR results in tumor cell cytosis (Hoover et al, 1995).

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Also, overreactions of the immune system in allergic reactions or due to massive antigen load might be reduced by, for example, intravenous application of soluble FcR (Ierino et al, 1993).

- 5 Therefore, a preferred pharmaceutical composition according to the invention for use in the treatment of AIDS, rheumatoid arthritis or multiple myeloma contains a recombinant soluble Fcy receptor and, preferably, a receptor having the amino acid sequence as shown in SEQ ID NO:1-4.
- 10 It was also of great interest to obtain crystal structure data of Fc receptors and/or Fc receptor/Ig complexes. On the one hand, these are a key to the understanding of molecular mechanisms in immunocomplex recognition. On the other hand, these structural data can be used to find out common features in the structures of different Fc receptors and use the knowledge 15 of the structures to generate inhibitors or identify and produce new artificial antibody receptors.

It was also of great interest to obtain information on the concrete binding sites of immunoglobulins to their respective receptors in naturally occurring 20 three-dimensional molecules. Therefrom even more precise findings on the interactions between antibody and receptor can be obtained and also on how these interactions can be modulated. In this connection modulation means either an enhancement of the interaction or a reduction leading to an inhibition by e.g. covering the binding sites on one or more parts of the 25 complex.

To obtain such crystal structure data and conformation information, a crystalline preparation of the recombinant soluble Fc receptor according to the invention is used. The recombinant soluble FcRs according to the 30 invention surprisingly can be obtained pure enough to produce crystals that give reliable X-ray structure determination data. Such crystallization was not

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possible with the hitherto produced receptor molecules, mostly due to their lack of homogeneity.

Therefore, another embodiment of the present invention concerns a crystalline preparation of an Fc receptor according to the invention. Yet another embodiment of the present invention is a crystalline preparation of a complex of soluble Fc receptor according to the invention together with the related immunoglobulin Fc part. Particulary preferred embodiments are shown in the examples as well as the relevant crystal structure data. Via crystal structure analysis of the crystalline preparations the exact amino acids of the Fc receptor/Ig complexes could be detected which mediate the coupling. These amino acids are in shown Fig 6a and 6b and the type of binding between the individual amino acids of both molecules in the complex is also indicated. A further embodiment of the present invention is therefore the use of a crystalline preparation of a recombinant soluble Fc receptor for the generation of crystal structure data of Fc receptors. From this crystal structure data information about the three-dimensional structure and the active sites for the binding of antibodies can be obtained. Especially preferably is the use of a crystalline preparation of a complex of recombinant soluble Fc receptor according to the invention and the corresponding immunoglobulin molecule for the generation of crystal structure data for the complexes. These data allow to determine the actual interactions that are formed between the two molecules and allow for the first time to obtain exact information about the interaction of the molecules thereby conferring knowledge about possible sites for inhibition or enhancement of the binding. On the basis of the information obtained from the crystal structure data the findings necessary for effecting modulation of the interaction between Fc receptor and immunoglobulin can be obtained. This modulation can be range from enhancement to complete inhibition to an inhibition of the binding.

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The stated applications are merely preferred embodiments of the use of the crystal structure data. Many other applications seem possible, too.

Suitably, the structural data for the generation and/or identification of 5 inhibitors or new receptors, respectively, are used in a computer-aided modelling program.

Particularly preferred for the present invention are the structures of FcRs or 10 FcR:Fc-fragment complexes as exemplified in figures and examples. Such structures can be used to design inhibitors, antagonists and artificial receptor molecules.

Computer programs suitable for computer-aided drug design and screening are known to the person skilled in the art and generally available. They 15 provide the possibility to examine umpteen compositions on the computer in view of their ability to bind to a certain molecule when the corresponding structure dates are entered in the computer. With the help of this possibility a great number of known chemical compositions can be examined regarding their inhibiting or antagonistic effect. The person skilled in the art merely 20 requires the crystal structure dates provided by the present invention and a commercially available screening program (Program Flexx: From the GMD-German National Research Center for Information Technology, Schloss Birlinghoven, D-53754 Sankt Augustin, Germany). A preferred embodiment of the present invention therefore is the use of the crystal structure data 25 obtained for the recombinant soluble Fc receptor according to the invention and for the complexes of recombinant soluble Fc receptor according to the invention and corresponding immunoglobulin in a computer aided modelling program for the identification and production of Fc receptor inhibitors.

30 Likewise, a further embodiment of the present invention is the use of the crystal structure data obtained for the receptors according to the invention and the receptor/immunoglobulin complexes, respectively for the

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identification and preparation of new Fc receptors which can be used, e.g. as antagonists and competitors. The crystal structure data and the data on the amino acids involved in the binding to Fc receptors obtained therefrom can serve for example to generate mutated immunoglobulins which can also 5 be used as inhibitors. It is imaginable that mutated or chemically modified inhibitors undergo tight binding and thus effect a blocking of receptors. On the other hand, the data obtained for the binding sites of immunoglobulins can also be used for the identification and/or preparation of inhibitors for 10 immunoglobulin molecules. Since the present invention teaches the binding sites to the receptor, it is easy to effect a blocking of the binding sites with the help of relatively simple molecules. Therefore, a further subject matter of the present invention is the use of the crystal structure data obtained for the FcR/Ig complexes for the identification and/or preparation of immunoglobulin inhibitors.

15

Accordingly, still further subject matter of the present invention are FcR inhibitors which have a three-dimensional structure which is complementary to the recombinant soluble FcR according to the invention and inhibit the binding of antibodies to FcRs.

20

Another further subject of the present invention are immunoglobulin inhibitors which have a three-dimensional structure which is complementary to the immunoglobulin binding site for recombinant soluble Fc receptors according to the invention and inhibit the binding of immunoglobulins to Fc 25 receptors.

The term "complementary" is to be understood within the framework of the invention in such a way that the inhibitor molecules must be substances which are able to cover at least so many binding sites on the 30 immunoglobulin or on the Fc receptor that the binding between Fc receptor and immunoglobulin is at least decisively weakened. Covering can take place both by binding to the amino acids mediating the complex formation

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of either component but also in such a way that at least complex formation is no longer possible, be it by sterically inhibition or by binding to adjacent amino acids, however, covering the amino acid involved in the complex binding between Fc receptor and immunoglobulin.

5

In connection with the present invention it was possible for the first time to determine the exact binding sites and the amino acids involved in the binding of the antibody and antibody receptor molecules. One is now able to design specifically binding molecules and to screen candidate 10 compositions on the computer. This enables the selection of such compositions from a variety of possibly candidate compositions which can effect a sufficient inhibition of complex formation between Fc receptor and immunoglobulin.

15 What is important for the inhibitors of the invention is that, owing to their structure and specificity, they are capable of binding to the FcRs or immunoglobulins and thus prevent the normal binding between FcRs and the constant parts of antibodies.

20 Preferably, such FcR or IgG inhibitors are small organic molecules which can easily be administered orally. They are an interesting alternative to cortisone in the treatment of autoimmune diseases and host/graf rejections. Such a molecule would also suppress reinfection rates with certain viruses, e.g. Dengue virus where the antibody coated virus is Fc $\gamma$ RIIb dependent 25 internalized (Littaua et al, 1990), HIV where on CD4 positive T cells an antibody enhancement of HIV infection is mediated by Fc $\gamma$ RIII (Homsy et al, 1989), or Ebola where the virus secreted glycoprotein inhibits early neutrophil activation by blocking sFc $\gamma$ RIII which affects the host response to infection (Yang et al, 1998).

30

The development of inhibitors also leads to substances that interfere with the recognition of IgE by their receptors. From the modelled structure of

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Fc $\epsilon$ RI, peptides have already been developed which inhibit mast cell degranulation in vitro. With the now available knowledge of the structures of the homologue receptors and the receptor-antibody complex in atomic detail, a new possibility for a rational drug design is opened.

5

The Fc-receptor bind between the two CH2-domains of the Fc-fragment in the so-called lower hinge region (Fig.8). The binding region of the Fc-receptor is described in Example 1 (The contact interface to IgG). The residues promoting the interaction between FcR and immunoglobulin are 10 shown in figures 7, 10a and 10b. Thereby three interaction regions become evident (Fig.5).

**1st region: FcR (residues 85 to 87 and residue 110) - Ig (Chain A residues 326-328)**

15 Proline 328 of the Ig is clamped by the residues Trp 87 and 110 in a sandwich like manner. These residues are conserved among the IgG and IgE receptors as well as in the IgG and IgE. An inhibitor binding to this prominent region would strongly interfere with binding. This region is additionally attractive for inhibitor design because the exposed hydrophobic 20 surface region comprising the residues Trp 87, Ile 85, Gly 86 of the receptors could be employed to obtain additional binding energy. The functional groups of Thr 113 and Glu 118 and Lys 119 side chains in the vicinity may contribute especially to specific inhibitor binding.

25 **2nd region: FcR (residues 126-132 and residues 155-158) - Ig (Chain A and Chain B residues 234-239)**

The amino terminal residues 234-239 of both Ig chains are recognised differently by the FcR, thereby breaking the 2-fold symmetry of the Fc fragment.

30 This residues of Fc-fragment chain A are in contact with residues Val 155 - Lys 158 of the receptor and the same residues from Fc-fragment chain B with receptor residues Gly 126 - His 132. This region shows the most

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differences in the sequence alignment of the receptors as well as the immunoglobulins and should therefore be involved in specificity generation. This deep cleft between the Fc-fragment chains is well suited for inhibitor design and would be the site of choice for the development of inhibitors  
5 when issues of specificity are concerned.

**3rd region: FcR (residues 117, 126 and 129-132) - Ig (Chain B residues  
264-265 and residues 296-297)**

This binding region is characterised by a clustering of amino acid residues  
10 carrying functional groups in their side chains, that might be employed in various ways for inhibitor design on the receptor and the Ig side of the contact.

Molecules that interact with one or more of the above described regions,  
15 and are designed or screened explicitly for exploiting the knowledge of binding sites are considered as inhibitors according to the invention.

Further subject matters of the present invention are pharmaceutical compositions containing as active agent an FcR inhibitor or an  
20 immunoglobulin inhibitor as mentioned above. Such pharmaceutical compositions may, for example, be used in the treatment or prevention of diseases which are due to overreactions or faulty reactions of the immune system, preferably the treatment or prevention of allergies, autoimmune diseases or anaphylactic shock.

25

A further subject of the present invention is the sFcR according to the invention, bound to a solid phase. Such heterogeneous receptors can be used for immunoassays or other applications where the receptor in an immobilized form can be used beneficially.

30

In a preferred embodiment of the invention the solid phase is a chromatography carrier material onto which the Fc receptor is fixed, e.g.

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sepharose, dextran sulfate etc. Such chromatography materials with Fc receptors bound thereto can beneficially be used for the adsorption of immunoglobulins from the blood, plasma or serum of patients or from the culture supernatant of immunoglobulin producing cells (meaning concentration, enrichment and purification of antibodies).

On the one hand, the antibodies bound to the chromatography material can be eluted and, for example, the immune status of a patient can thereby be determined. On the other hand, antibodies from the blood of a patient can thereby be enriched before carrying out further tests, which is a further preferred embodiment of the present invention. In many cases it is difficult to conduct diagnostic assays using blood samples if the latter contains only a very small number of the antibodies to be identified. By means of a concentration using a specific chromatographic column with Fc receptors according to the present invention, antibodies of interest can easily be concentrated and separated from many other substances which might disturb the test.

Basically, it is also possible to use a chromatography material according to the present invention in an extracorporeal perfusion system for lavage of the blood in case of certain diseases where the removal of antibodies plays a crucial role.

It is, however, also possible to use another material as solid phase to which the soluble Fc receptor according to the invention is coupled, e.g. microtiter plates or small reaction vessels to the walls of which Fc receptors are bound either directly or indirectly. Such solid phases and vessels can be particularly important for diagnostic methods, as they enable screening by using immunoassays e.g. for detecting the presence of certain immunoglobulins in patients' blood or other body fluids.

To sum up, the recombinant soluble Fc receptors provided by the present invention as well as the corresponding structure determination of crystalline

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preparations of these receptors and of crystalline complexes of receptors and immunoglobins enable for the first time to perform a rational drug design, wherefrom it is possible to modulate the interaction between immunoglobulins and Fc receptors on cells or soluble receptors. Such a 5 modulation is preferably an inhibition, whereby the inhibition of the formation of a complex from IgG and Fc receptor takes place by covering and preferably by binding of inhibitor molecules to the Fc receptor or the immunoglobulin. There are various medical applications for such modulating drugs and in particular of inhibitors and only few of these applications have 10 been exemplary mentioned within the framework of the present specification. This can and should by no means exclude the applicability of such molecules which have been designed or screened on the basis of the findings about the molecular structure or FcR/Ig complexes disclosed herein for the treatment or prevention of other health disturbances.

15

The following Examples are to further illustrate the invention in conjunction with the Figures.

#### Example 1

20 **shFcγRIIb (soluble human FcγRIIb)**

##### **1.1 Cloning and Expression**

The cDNA of human FcγRIIb2 (Engelhardt et al, 1990) was modified using 25 mutagenous PCR (Dulau et al, 1989). Therefore, a forward primer was used for the introduction of a new start methionine after the cleavage site of the signal peptide within a *N*col site (5'-AAT AGA ATT CCA TGG GGA CAC CTG CAG CTC CC-3') while the reverse primer introduced a stop codon between the putative extracellular part and the transmembrane region 30 followed by a *S*a/l site (5' CCC AGT GTC GAC AGC CTA AAT GAT CCC C-3'). The PCR product was digested with *N*col and *S*a/l, cloned into a pET11d expression vector (Novagen) and the proposed sequence was

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confirmed. The final construct was propagated in BL21(DE3) (Grodberg and Dunn, 1988). For the overexpression of Fc $\gamma$ RIIb a single colony of the transformed bacteria was inoculated in 5ml LB medium containing 100  $\mu$ g ampicillin per ml (LB-Amp100) and incubated overnight at 37°C. The 5 culture was diluted 200-fold in LB-Amp100 and incubation was continued until an OD600 of 0.7-0.9 was achieved. The overproduction of the protein was induced by adding IPTG to a final concentration of 1 mM. After a growing period of 4 hours the cells were harvested by centrifugation (30 min, 4000 x g) and resuspended in sonification buffer (30 mM sodium 10 phosphate, 300 mM sodium chloride, 0.02% sodium azide, pH 7.8). After addition of 0.1 mg lysozyme per ml suspension and incubation for 30 min at room temperature the sonification was performed on ice (Branson Sonifier, Danbury, CT; Macrotip, 90% output, 80% interval, 15 min). The suspension was centrifuged (30 min, 30,000 x g) and resuspended with a 15 Dounce homogenizer in sonification buffer containing 0.5% LDAO. The centrifugation step and resuspension in LDAO containing buffer was repeated once before this procedure was repeated twice without LDAO. The purified inclusion bodies were stored at 4°C.

20      **1.2 Refolding and purification of soluble human Fc $\gamma$ RIIb (shFc $\gamma$ RIIb)**

The purified inclusion bodies were dissolved to a protein concentration of 10 mg/ml in 6 M guanidine chloride, 100 mM 2-mercaptoethanol and separated from the insoluble matter by centrifugation. The refolding was 25 achieved by rapid dilution. Therefore, one ml of the inclusion body solution was dropped under stirring within 15 hours into 400 ml of the refolding buffer (0.1 M TRIS/HCl, 1.4 M arginine, 150 mM sodium chloride, 5 mM GSH, 0.5 mM GSSG, 0.1 mM PMSF, 0.02% sodium azide, pH 8.5, 4°C). Afterwards, the mixture was stirred for 2-3 days until the concentration of 30 free thiol groups was reduced to 1 mM by air oxidation as measured according to Ellman (Ellman, 1959). The solution was dialyzed against PBS and sterile filtered before it was concentrated 10-fold in a stirring cell

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equipped with a 3kD MWCO ultrafiltration membrane. The protein solution was applied to a hlgG sepharose column (50 mg hlgG per ml sepharose 4B). Unbound protein was washed out with 50 mM TRIS pH 8.0 before elution of Fc $\gamma$ RIIb by pH jump (150 mM sodium chloride, 100 mM glycine, 0.02% sodium azide, pH 3.0). The eluate was immediately neutralized with 1 M TRIS pH 8.0. The Fc $\gamma$ RIIb containing solution was concentrated and subjected to gel filtration on a Superdex-75 column equilibrated with crystallization buffer (2 mM MOPS 150 mM sodium chloride, 0.02% sodium azide pH 7.0). The fractions containing Fc $\gamma$ RIIb were pooled, concentrated to 7 mg/ml and stored at -20°C.

### 1.3 Equilibrium gel filtration experiments

A Superdex75 column was connected to FPLC and equilibrated with PBS containing 10  $\mu$ g shFcRIIb per ml. Human Fc fragment was solved to a concentration of 1  $\mu$ g/10  $\mu$ l in the equilibration buffer and injected. The resulting chromatogram yielded a positive peak comprising the complex of the shFc $\gamma$ RIIb and the Fc fragment while the negative peak represents the lack of receptor consumed from the running buffer for complex formation.

20

### 1.4 Crystallization and data collection

Initial crystallization trials employing a 96 condition sparse matrix screen (Jancarik and Kim, 1991) were performed in sitting drops at 20 °C using the vapor diffusion method. Occuring crystals were improved by changing the pH as well as the salt, precipitant and additive concentration. Diffraction data from suitable crystals was collected on an image plate system (MAR research) using graphite monochromated CuK $\alpha$  radiation from a RU200b rotating anode generator (Rigaku) operated at 50 kV and 100 mA. The reflections were integrated with the program MOSFLM (Leslie, 1997) and subsequently the data was scaled, reduced and truncated to obtain the

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structure-factor amplitudes using routines from the CCP4 program suite (Collaborative Computational Project, 1994).

### 1.5 Summary of expression, purification and refolding of shFc $\gamma$ RIIb

5

The extracellular part of Fc $\gamma$ RIIb was expressed in high levels under the control of a T7 promoter in the T7 RNA polymerase positive E. coli strand BL21/DE3 (Grodberg & Dunn, 1988). The protein was deposited in inclusion bodies, which were employed in the first purification step. The isolation of 10 the inclusion bodies was started with an intense combined lysozyme/sonification procedure to open virtually all cells which would otherwise contaminate the product. The subsequent washing steps with the detergent LDAO, which has excellent properties in solving impurities but not the inclusion bodies itself already yielded a product with a purity of > 90% (Fig. 15 1).

This product was used for refolding trials without further purification. The inclusion bodies were dissolved in high concentration of 2-mercaptoethanol and guanidine to ensure the shift of covalent and non-covalent aggregates 20 to monomers. This solution was rapidly diluted with refolding buffer to minimize contacts between the unfolded protein molecules which would otherwise form aggregates. The use of arginine in the refolding buffer prevents the irreversible modification of side chains as often recognized with urea. After addition of the protein to the refolding buffer, the solution 25 was stirred at 4 °C until the concentration of free thiol groups was reduced to 1 mM, which was absolutely necessary as earlier dialysis resulted in an inactive product. In a second purification step the dialyzed and refolded Fc $\gamma$ RIIb was bound to immobilized hIgG to remove minor fractions of E. coli proteins and inactive receptor. The protein was eluted with a pH jump and 30 immediately neutralized. After this affinity chromatography step shFc $\gamma$ RIIb is essentially pure except for a minor contamination resulting from the coeluting IgG which leached out of the matrix even after repeated use (Fig.

- 21 -

- 1). The IgG as well as receptor multimers which are not visible in the reducing SDS-PAGE could easily be removed by gel filtration. Parallel to the removal of the contaminants in this step the buffer is quantitatively exchanged. This procedure ensures a defined composition of the protein solution as even slight variations can cause irreproducibility of the crystallization attempts or even inhibit the formation of crystals. Overall 6 mg pure protein could be gained per litre E. coli culture, which is about 10 % from the Fc<sub>y</sub>RIIb content of the inclusion bodies.
- 5
- 10 N-terminal protein sequencing revealed the identity with the expected sequence H<sub>2</sub>N-GTPAAP without detectable contamination. ESI-MS analysis showed that the final material used in crystallization trials is homogenous with respect to size. From the primary sequence the molecular weight was calculated to 20434 Da, which corresponds to 20429 Da found by mass spectroscopy. The discrepancy lies within the error of the instrument, and 15 no additional peak for a species containing the leading methionine is found.

The crystallization of shFc<sub>y</sub>RIIb was performed in sitting drops using the vapor diffusion method. Initial trials with a sparse matrix screen (Jancarik & Kim, 1991) resulted already in small crystalline needles. Subsequent 20 optimization of the preliminary crystallization condition by varying precipitant, salt, their concentration and pH led to the isolation of three different crystal forms. Orthorhombic crystals grew from mixture of 1.5 µl reservoir solution (33% PEG2000, 0.2 M sodium acetate, pH 5.4) with 3 µl of the protein solution. They appeared within 3 days and reached their 25 final size of approximately 80 µm x 80 µm x 500 µm after one week. These crystals diffracted to 1.7 Å. Crystals could also be grown in two other space groups from reservoir solution containing 26% PEG8000, 0.2 M sodium acetate, pH 5.6, 5 mM Zn(OAc)<sub>2</sub>, 100 mM sodium chloride 30 (hexagonal form) and 26% PEG8000, 0.2 M NaOAc, pH 5.6, 10% (v/v) 1,4-Dioxan, 100 mM sodium chloride (tetragonal form). These crystals were

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of suitable size for X-ray analysis but diffracted only to 2.7 Å and 3.8 Å for the tetragonal and hexagonal crystal form respectively (Table 1).

FcyRII was expressed in *E. coli* which, besides the comparatively low production costs and the availability, has several advantages especially when the glycosylation performed by mammalian cells is not necessary for the function of the protein as in the case of FcyRII where IgG binding occurs independently of carbohydrate attachment (Sondermann et al, 1998A). In *E. coli* a homogenous product can reproducibly be generated, which is in contrast to the expression in mammalian cells where batch dependent variances are often observed. In such a system the product is for several days exposed to proteases at temperatures of more than 30 °C. In contrary, the expression of the protein in *E. coli* under the control of the strong T7 promoter at 37 °C frequently leads to the formation of protease inaccessible inclusion bodies. A further advantage of the expression in bacteria is that the material could be considered to be free of pathogenic germs, which might derive from employed fetal calf serum or the cell line itself. In mammalian expression particular care must be taken during the purification of the target protein because potential effective hormones or growth factors might be copurified. One case where the effects of sFcyR were ascribed to a TGF $\beta$ 1 contamination is already reported (Galon et al, 1995).

### 1.6 Purification

25

The purification procedure is straightforward. It consists of three steps which can easily be performed in a single day. The protein is obtained in a pure form and in high yields and could even be obtained in considerable quality without the expensive IgG affinity column. The success of such a protocol would depend on the careful preparation of the inclusion bodies, as most of the impurities can be eliminated already in the first purification step.

### 1.7 Characterization

The purified Fc $\gamma$ RIIb was characterized by SDS-PAGE and isoelectric focussing as well as N-terminal sequencing and mass spectroscopy. Thus,  
5 the material can be considered pure and homogeneous with respect to its chemical composition, but the intriguing question whether the receptor is correctly folded remains to be discussed. All cysteins are paired, since no free thiol groups are detected with Ellman's test. The material is monomeric and elutes with the expected retention time in peaks of symmetrical shape  
10 from a size exclusion chromatography column. Furthermore, Fc $\gamma$ RIIb binds to IgG sepharose, recombinant Fc $\gamma$ RIIb from *E. coli* is active because it specifically binds IgG.

### 1.8 Crystallization

15

The orthorhombic crystal form of Fc $\gamma$ RIIb diffracted X-rays to a resolution of 1.7 Å, which is a drastic improvement compared to previously reported crystals of the same molecule derived from insect cell expression (Sondermann et al, 1998A). These crystals diffracted to 2.9 Å and were of  
20 space group P3,21. Thus, the glycosylation of the insect cell derived receptor influences the crystallization conditions. Instead of the trigonal space group, three different crystal forms are found. After a possible solution of the structure these crystal forms will help identify artificial conformations of the protein due to crystal contacts.

25

Fc $\gamma$ Rs do not exhibit any sequence similarity to other proteins but due to a conserved cystein spacing they are affiliated to the immunoglobulin super family. Consequently, we tried to solve its structure by molecular replacement, but extensive trials using IgG domains from a variety of  
30 molecules failed. Thus the structure of Fc $\gamma$ RIIb has to be solved by the methods of multiple isomorphous replacement.

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We have shown for the first time that Fc $\gamma$ RIIb can be obtained in an active form from *E. coli*. This is the basis for crystallographic investigations that will soon, due to the already gained crystals of exceptional quality, result in the structure solution of this important molecule. The structure will 5 provide information on the IgG binding site and provide a starting point for the knowledge based design of drugs that interfere with recognition of the ligand by its receptor. Furthermore, because of the high homology between Fc $\gamma$ RIIb and other FcRs including Fc $\epsilon$ RIa it seems possible that these molecules can be produced in the same way, which would provide valuable 10 material for the ongoing research.

### 1.9 Methods

#### Protein chemistry

15 Recombinant soluble human Fc $\gamma$ RIIb was expressed in *E.coli*, refolded purified and crystallized as described elsewhere (Sondermann et al, 1998B). Briefly, the putative extracellular region of hFc $\gamma$ RIIb2 (Engelhardt et al, 1990) was overexpressed in *E. coli*. Inclusion bodies were purified by 20 lysozyme treatment of the cells and subsequent sonification. The resulting suspension was centrifuged (30 min 30,000 x g) and washed with buffer containing 0.5% LDAO. A centrifugation step and resuspension in LDAO containing buffer was repeated once before this procedure was repeated twice without LDAO. The inclusion bodies were solved in 6 M guanidine hydrochloride and the protein was renatured as described. The dialyzed 25 and filtrated protein solution was applied to a hIgG sepharose column and eluted by pH jump. The concentrated neutralized fractions were subjected to size-exclusion chromatography on a Superdex-75 column (26/60, Pharmacia).

#### 30 Crystallization

Crystallization was performed in sitting drops at 20°C using the vapor diffusion technique. Crystallization screens were performed by changing pH,

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salt, precipitant and additives. The final crystals used for data collection were grown in 33% PEG2000, 0.2 M sodium acetate, pH 5.4 (orthorhombic form) 26% PEG8000, 0.2 M sodium acetate, pH 5.6, 10% (v/v) 1,4-dioxane, 100 mM sodium chloride (tetragonal form), and 26% 5 PEG8000, 0.2 M sodium acetate, pH 5.6, 5mM ZN(OAc)<sub>2</sub>, 100 mM sodium chloride (hexagonal form). The insect cell derived protein was crystallized in 32% PEG6000, 0.2 M sodium acetate, pH 5.3.

#### **Preparation of heavy-atom derivatives**

10 The heavy-atom derivatives were prepared by soaking the crystals in the crystallization buffer containing 2 mM platinum(II)-(2,2'-6,2''terpyridinium) chloride for 24 hours or 10 mM uranylchloride for 8 days.

#### **X-ray data collection**

15 Diffraction data was collected on an image plate system (MAR research) using graphite monochromated CuK<sub>α</sub> radiation from a RU200b rotating anode generator (Rigaku) operated at 50 kV and 100 mA. The reflections were integrated with the program MOSFLM 5.50 (Leslie, 1997) and subsequently the data was scaled and truncated to obtain the 20 structure-factor amplitudes using routines from the CCP4 program suite (Collaborative Computational Project, 1994).

#### **Structure determination**

The structure was solved with the standard procedures of the MIR method.  
25 From the large number of soaks carried out with different heavy-atom components only the two compounds yielded interpretable Patterson maps. The heavy-atom positions for each derivative were determined from difference Patterson maps and initial phases were calculated. Cross-phased difference Fourier maps were used to confirm heavy atom positions and 30 establish a common origin for the derivatives. Anomalous data were included to discriminate between the enantiomers. The heavy atom parameters were further refined with the program MLPHARE from the CCP4

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package leading to the statistics compiled in Table 2. An electron-density map was calculated to a resolution of 2.1 Å and the phases were improved further by solvent flattening and histogram matching with the program DM from the CCP4 suite. The resulting electron density map was of sufficient quality to build most of the amino acid residues. Model building was performed with O (Jones et al, 1991) on an Indigo2 work station (Silicon Graphics Incorporation). The structure refinement was done with XPLOR (Brünger et al, 1987) by gradually increasing the resolution to 1.7 Å using the parameter set of Engh and Huber (Engh & Huber, 1991). When the structure was complete after several rounds of model building and individual restraint B-factors refinement ( $R_{\text{fac}} = 29\% / R_{\text{free}} = 36\%$ ), 150 water molecules were built into the electron density when a Fo-Fc map contoured at 3.5 σ coincided with well defined electron density of a 2Fo-Fc map contoured at 1 σ. The resulting refinement statistic is shown in Table 3.

15

### 1.10 Structure determination

The crystal structure of recombinant soluble human FcγRIIb was solved by multiple isomorphous replacement (MIR) to 1.7 Å resolution, since a structure solution by molecular replacement with isolated domains of the Fc fragment from human IgG1 (Huber et al, 1976, PDB entry 1fc1; Deisenhofer, 1981) failed. The putative extracellular part of the receptor (amino acid residues 1-187 as depicted in SEQ ID NO:2) was used for crystallization trials (Sondermann et al, 1998B) while the model contains the residues 5-176 as the termini are flexible and not traceable into the electron density. Additionally, the model contains 150 water molecules and the refinement statistics are summarized in Table 2. The structure contains a cis proline at position 11. None of the main chain torsion angles is located in disallowed regions of the Ramachandran plot. The fully refined model was used to solve the structure of the same protein in crystals of space group P4<sub>2</sub>2<sub>1</sub>2 and of the glycosylated form derived from insect cells in crystals of space group P3<sub>1</sub>21 (Table 2).

The polypeptide chain of Fc $\gamma$ RIIb folds into two Ig-like domains as expected from its affiliation with the immunoglobulin super family. Each domain consists of two beta sheets that are arranged in a sandwich with the conserved disulfide bridge connecting strands B and F on the opposing sheets (Fig. 3). Three anti-parallel  $\beta$ -strands (A1, B, E) oppose a sheet of 5  $\beta$ -strands (C', C, F, G, A2), whereby strand A1 leaves the 3-stranded  $\beta$ -sheet and crosses over to the 4-stranded anti-parallel sheet adding the short parallel 5th strand A2. The arrangement of secondary structure elements as well as their connectivity is identical in both domains of the 10 Fc $\gamma$ RIIb and a rigid body fit of one domain onto the other revealed a r.m.s. distance of 1.29  $\text{\AA}$  of 67 matching  $\text{C}\alpha$  atoms.

The domains are arranged nearly perpendicularly to each other enclosing an angle of 70 degrees between their long axes forming a heart-shaped overall 15 structure. This arrangement results in an extensive contact region between the domains (Fig. 4). Residues from strand A2 and from the segment linking A2 and A1 of the N-terminal domain intermesh with residues of strands A1 and B from the C-terminal domain. This region is tightly packed and the interaction is strengthened by several hydrogen bonds resulting in a rigid 20 arrangement. This is confirmed by the conservation of the structure in three different space groups. In orthorhombic, tetragonal and hexagonal (insect cell derived) crystal forms a deviation of less than 2° in the interdomain angle is found.

### 25 1.11 Overall structures

The structure of recombinant human Fc $\gamma$ RIIb derived from E.coli was solved by MIR to 1.7  $\text{\AA}$  resolution from orthorhombic crystals. An essentially 30 identical structure is found in tetragonal and with protein derived from insect cells in hexagonal crystals. In all three structures the last nine residues of the polypeptide chain were found disordered. The flexibility of the C-terminal linker region between the structured core of the molecule and

the transmembrane part may be functionally relevant to allow some reorientation of the receptor to enhance the recognition of the Fc parts in immunocomplexes.

5      **1.12 Homologue receptors**

The Ig domains found in the Ig super family of proteins are characterized by a beta sandwich structure with a conserved disulfide bridge connecting two strands of the opposing sheets. The typical arrangement of 3 and 4 anti parallel beta strands that form a sandwich as found in Fc $\gamma$ RIIb occurs also in the T cell receptor, Fc fragment, CD4 or the Fab fragment. A structural alignment of the individual Ig domains of these molecules with the two domains of Fc $\gamma$ RIIb shows a common, closely related structure. The relative arrangement of the domains, however, is not related in these molecules and covers a broad sector. Despite the structural similarity between Ig domains from different molecules and the strikingly low r.m.s. deviation of C $\alpha$  atoms that result when the two domains of Fc $\gamma$ RII are superimposed, no significant sequence similarity is found (Figs. 5a and 5b). A structure-based sequence alignment shows a conserved hydrophobicity pattern along the sequence of the domains, together with, beside the cysteins, only few identical amino acid residues. We first prepared a structure-based alignment of the two C-terminal domains of the IgG1 heavy chain and the Fc $\gamma$ RIIb and added the sequences of the other related Fc $\gamma$ R and the Fc $\epsilon$ RIa domains. This shows that the sequences of the three domain Fc $\gamma$ RI and the two domain receptors are compatible with the hydrophobicity pattern of Ig domains and several conserved amino acid residues are revealed. Firstly, the different domains of an FcR are more related to each other than to Ig domains from other molecules of the Ig super family. Secondly, the N-terminal domains of the receptors relate to each other as the second domains do. Thirdly, the sequence of the third domain of Fc $\gamma$ RI shows features from both groups of domains. Taken together, we confirm the affiliation of the FcRs to the Ig super family and speculate that all FcR-domains originate from a common

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ancestor, an ancient one domain receptor that acquired a second domain by gene duplication. Further divergent development of such a two domain receptor resulted in the present diversity, including Fc $\gamma$ RI that acquired a third domain.

5

Conservation of these amino acid residues that contribute to the interdomain contact in Fc $\gamma$ RIIb in the alignment are a hint to a similar domain arrangement in different receptors. In Table 4 the residues contributing with their side chains to the interdomain contact (Fig. 4) are 10 compiled for Fc $\gamma$ RIIb together with the corresponding amino acid residues in other receptors according to the structure-based sequence alignment of Fig. 5b. Except for Asn15, which is not conserved between the FcRs, the involved residues are identical or conservatively replaced providing strong support for a similar structure and domain arrangement in all FcRs.

15

### 1.13 The contact interface to IgG

Limited information about the interactions of FcRs with their ligands is available from mutagenesis studies (Hogarth et al, 1992; Hulett et al, 1994; 20 Hulett et al, 1995). By systematically exchanging loops between the  $\beta$ -strands of Fc $\gamma$ RIIa for Fc $\epsilon$ RIa amino acid residues the B/C, C'/E and F/G loops of the C-terminal domain were evaluated as important for ligand binding (Fig. 3, Fig. 5b). In the structure model these loops are adjacent and freely accessible to the potential ligand. Additionally, most of the amino 25 acid residues in these loops were exchanged for alanines by single site mutations which resulted in a drastic alteration of the affinity of Fc $\gamma$ RIIa to dimeric human IgG1. Also, the single amino acid exchange Arg 131 to His in the C-terminal domain (C'/E loop) in the high responder/low responder polymorphism, which alters the affinity of the Fc $\gamma$ RIIa to murine IgG1, 30 points to that region. Thus, the amino acid residues in this area are either important for ligand binding or the structural integrity of that region. Here, the structure shows a clustering of the hydrophobic amino acid residues Pro

- 30 -

114, Leu 115 and Val 116 in the neighbourhood of Tyr 157. This patch is separated from the region Leu 159, Phe 121 and Phe 129 by the positively charged amino acid residues Arg 131 and Lys 117 which protrude from the core structure (Fig. 5b).

5

#### 1.14 Glycosylation

In the sequence of Fc $\gamma$ RIIb three potential N-glycosylation sites are found. All three sites are on the surface of the molecule and are accessible. They  
10 are located in the E/F loops (N61 and N142) of both domains and on strand E (N135) of the C-terminal domain (Fig. 3, Fig. 6). Since the material used for the solution of this structure was obtained from *E. coli*, it does not contain carbohydrates, while the FcRs isolated from mammalian cells are highly glycosylated. The three potential glycosylation sites are located  
15 rather far from the putative IgG binding region, and non-glycosylated Fc $\gamma$ RIIb binds human IgG, suggesting a minor role of glycosylation in binding. This was confirmed by the structure of the Fc $\gamma$ RIIb produced in insect cells which is glycosylated (Sondermann et al, 1998A). Except for a  
20 2° change of the interdomain angle possibly due to different crystal contacts, no differences between the glycosylated and unglycosylated protein structures were found. The three glycosylation sites are only optionally used as shown by SDS-PAGE where the material appears in 4 bands. No additional electron density for those sugars was found a consequence of chemical and structural heterogeneity.

25

#### Example 2

##### shFc $\gamma$ RIIa (soluble human Fc $\gamma$ RIIa)

The procedures were performed according to example 1 except for the indicated changes:

30

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### 2.1 Cloning and Expression

shFc $\gamma$ RIIa was generated by mutating the respective wild-type cDNA (Stengelin et al., 1988) and expressed according to example 1 with the mutagenous primers listed in table 5. For the expression of the protein a pET22b + vector was chosen.

### 2.2 Refolding and purification

shFc $\gamma$ RIIa was refolded according to example 1 with the respective refolding buffer listed in table 6.

10

### 2.3 Crystallisation

shFc $\gamma$ RIIa was crystallised as described under conditions indicated in table 7.

15

### 2.4 Structure determination

The structure was solved with the method of isomorphous replacement with shFc $\gamma$ RIIb as search model.

### Example 3

20

### shFc $\gamma$ RIII (soluble human Fc $\gamma$ RIII)

The procedure was performed according to example 1 except for the indicated changes:

25

### 3.1 Cloning and Expression

30

shFc $\gamma$ RIII was generated by mutating the respective wild-type cDNA (Simmons & Seed, 1988) and expressed according to example 1 with the mutagenous primers listed in table 5. For the expression of the protein a pET22b + vector was chosen.

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### 3.2 Refolding and purification

shFc $\gamma$ RIII was refolded according to example 1 with the respective refolding buffer listed in table 6.

5      **3.3 Crystallisation**

shFc $\gamma$ RIII was crystallised as described under conditions indicated in table 7.

### 3.4 Structure determination

10     The structure was solved with the method of isomorphous replacement with shFc $\gamma$ RIIb as search model.

### 3.5 Crystallisation of a shFc $\gamma$ RIII:hFc1 complex

15     hIgG1 derived from the serum of a myeloma patient was used to prepare Fc-fragments (hFc1) by digestion with plasmin (Deisenhofer et al., 1976).

The resulting Fc-fragments were separated from the Fab-fragments by protein A chromatography. Partially digested hIgG was removed by size exclusion chromatography with MBS (2mM MOPS, 150mM NaCl, 0.02% sodium azide, pH 7.0) as running buffer. Equimolar amounts of hFc1 and 20 shFc $\gamma$ RIII were mixed and diluted with MBS to a concentration of 10mg/ml.

The complex was crystallised as described under conditions indicated in table 5.

### Example 4

25     **shFc $\epsilon$ RII (soluble human Fc $\epsilon$ RII)**

The procedure was performed according to example 1 except for the indicated changes:

30     **4.1 Cloning and Expression**

Fc $\epsilon$ RII was generated by mutating the respective wild-type cDNA (Kikutani et al., 1986) and expressed according to example 2 with the mutagenous

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primers listed in table 5. For the expression of the protein a pET23a+ vector was chosen.

#### 4.2 Refolding and purification

Refolding of shFc $\epsilon$ RII was achieved as described in example 1, with the exception that prior to rapid dilution the dissolved inclusion bodies were dialysed against 6M guanidine chloride, 20mM sodium acetate, pH 4.0. shFc $\epsilon$ RII was refolded according to example 1 with the respective refolding buffer listed in table 6. After refolding the protein solution was dialysed against PBS, concentrated 100-fold and purified by gel filtration chromatography on Superdex 75. This yielded pure shFc $\epsilon$ RII which was dialysed against 2mM TRIS/HCl, 150mM NaCl, 0.02% sodium azide, pH 8.0, concentrated to 10mg/ml and stored at 4°C.

15 **Example 5**

**shFc $\gamma$ RI (soluble human Fc $\gamma$ RI)**

The procedure was performed according to example 1 except for the indicated changes:

20

#### 5.1 Cloning and Expression

shFc $\gamma$ RI was generated by mutating the respective wild-type cDNA (Allen & Seed, 1988) and expressed according to example 1 with the mutagenous primers listed in table 5. For the expression of the protein a pET32a+ vector was chosen, which contains after the N-terminal thioredoxin a hexahistidine-tag with a C-terminal thrombin cleavage site followed by the shFc $\gamma$ RI in frame with the mentioned proteins and amino acid residues. For the overexpression of the fusion protein the E.coli strain BL21(DE3) containing the plasmids pUBS and pLysS (Novagen) was used.

30

The purified inclusion bodies were solubilised in 6M guanidine-HCl, 10mM  $\beta$ -mercaptoethanol, 50mM Tris pH8.0 and bound to a Ni-NTA column

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(Qiagen). The elution was performed with an imidazole gradient ranging from 0 to 1M imidazole. The eluted protein was dialysed against a 1000fold volume of 150mM NaCl, 50mM Tris pH8.0, 2mM GSH, 0.5mM GSSG for 24 hours at 4°C. After concentrating the protein solution to 25% of the initial volume, thrombin was added. After 6h of incubation at 37°C the N-terminal thioredoxin and the His-tag were removed completely as verified by N-terminal sequencing. During this digestion the shFc<sub>g</sub>RI precipitated quantitatively out of solution.

10 **5.2 Refolding and purification**

shFc<sub>g</sub>RI was refolded according to example 1 with the respective refolding buffer listed in table 6. After the redox potential decresased to 1mM the solution was dialysed against PBS pH8.0 and concentrated.

15 The refolded Protein was analysed by size exclusion chromatography, which yielded a peak of the proposed monomeric receptor and non reducing SDS-PAGE which showed a major band at 30kDa.

**Example 6**

20 **shFc<sub>ε</sub>RIa (soluble human Fc<sub>ε</sub>RIa)**

The procedure was performed according to example 1 except for the indicated changes:

25 **6.1 Cloning and Expression**

shFc<sub>ε</sub>RI was generated by mutating the respective wild-type cDNA (Kochan et al., 1988) and expressed according to example 1 with the mutagenous primers listed in table 5. For the expression of the protein a pET23a+ vector was chosen.

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#### Brief description of the figures

##### **Fig. 1: 15% reducing SDS PAGE showing the purification of sFc $\gamma$ RIIb**

Lane 1: Molecular weight marker. Lane 2: E. coli lysate before induction.

5 Lane 3: E. coli lysate 1 h after induction. Lane 4: E.coli lysate 4 h after induction. Lane 5: Purified inclusion bodies of sFc $\gamma$ RIIb. Lane 6: Eluate of the hlgG affinity column. Lane 7: Pooled fractions of the gel filtration column.

##### **Fig. 2: Equilibrium gel filtration**

10 1  $\mu$ g hFc solved in 10  $\mu$ l equilibration buffer (10  $\mu$ g sFc $\gamma$ RIIb/ml PBS) was applied to a size exclusion chromatography column and the absorbance of the effluent was measured (280 nm) as a function of time. The injected Fc fragment forms a complex with the sFc $\gamma$ RIIb in the equilibration buffer (t = 22min). The negative peak of consumed sFc $\gamma$ RIIb is observed at t = 26 min.

##### **Fig. 3: Overall structure of human sFc $\gamma$ RIIb**

Stereo ribbon representation of the sFc $\gamma$ RIIb structure. The loops supposed 20 to be important for IgG binding are depicted in red with some of the residues within the binding site and the conserved disulfide bridge in ball and stick representation. The potential N-glycosylation sites are shown as green balls. The termini are labeled and the  $\beta$ -strands are numbered consecutively for the N-terminal domain in black and for the C-terminal 25 domain in blue. The figure was created using the programs MOLSCRIPT (Kraulis, 1991) and RENDER (Merritt and Murphy, 1994).

##### **Fig. 4: Interdomain contacts**

The figure shows a close-up on the residues involved in the interdomain 30 contacts of sFc $\gamma$ RIIb. The amino acid residues of the N-terminal domain are depicted blue and the residues of the C-terminal domain yellow. The model is covered by a 2Fo-Fc electron density contoured at 1  $\sigma$  obtained from the

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final coordinates. Hydrogen bridges between the domains are represented by white lines. The figure was created using the program MAIN (Turk, 1992).

5      **Fig. 5a: Superposition of the two Fc $\gamma$ RIIb domains and the CH2 domain of human IgG1**

Both domains of Fc $\gamma$ RIIb and the CH2 domain of hIgG1 were superimposed. The N-terminal domain is depicted in blue, the C-terminal domain in red and the CH2 domain of hIgG1 in green. The respective termini are labeled and 10 the conserved disulfide bridges are depicted as thin lines.

**Fig. 5b: Structure based sequence alignment of the sFc $\gamma$ RIIb domains with domains of other members of the FcR family**

The upper part of the figure shows the structure based sequence alignment 15 of the Fc $\gamma$ RIIb and hIgG1 Fc fragment domains performed with the program GBF-3D-FIT (Lessel & Schomburg, 1994). Amino acid residues with a  $C\alpha$  distance of less than 2.0 Å in the superimposed domains are masked: lilac for matching residues between the Fc fragment domains; yellow for residues in the Fc $\gamma$ RIIb domains; and green when they can be superimposed 20 in all four domains. The  $\beta$ -strands are indicated below this part of the alignment and are labeled consistent with Figure 3.

The lower part of the figure shows the alignment of the amino acid 25 sequences from the other Fc $\gamma$ Rs and the homologue Fc $\epsilon$ RIa to the profile given in the upper part of the figure using routines from the GCG package (Genetics Computer Group, 1994). The upper and lower row of numbering refer to the N- and C-terminal domains of Fc $\gamma$ RIIb. The conserved cysteins 30 are typed in magenta and the potential glycosylation sites in blue. Identical residues within the first domain are masked orange, those in the second domain pink and green when the residues are conserved within both domains. The less conserved third domain of Fc $\gamma$ RI is aligned between the first and the second domains. Red arrows point to residues that are involved

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in side chain contacts between the first and the second domain while blue arrows depict residues that are relevant for IgG binding. The figure was produced with the program ALSCRIPT (Barton, 1993).

5      **Fig. 6: The putative binding sites of Fc $\gamma$ RIIb**

Solid surface representations of Fc $\gamma$ RIIb as produced with GRASP (Nicholls et al, 1991), the color coding is according to the relative surface potential from negative (red) to positive (blue). Fig. 6a shows the molecule as in Fig. 10      3 by a rotation of about 90° counter-clockwise around the vertical. In Fig. 6b the molecule is rotated 90° clockwise around the same axis. Both views show the putative binding regions on the C-terminal (Fig. 6a) and the N-terminal domain (Fig. 6b). The amino acid residues discussed in the text are labeled.

15     **Fig. 7:  $\alpha$ -trace of the superpositioned structures of the Fc $\gamma$ -receptors**  
Fc $\gamma$ RIII red, Fc $\gamma$ RIIa green and Fc $\gamma$ RIIb blue. Residues important for IgG binding are shown in ball-and-stick. The N- and C-termini are labelled.

20     **Fig. 8: Overview of the Fc $\gamma$ RIII/Fc-fragment crystal structure in ribbon representation**

The sugar residues bound to the Fc-Fragment are indicated in ball-and-stick. The Fc $\gamma$ RIII (blue) binds in the lower hinge region between chain-B (red) and chain-A (green) of the Fc-fragment.

25     **Fig. 9: Close-up on the binding region of the Fc $\gamma$ RIII and the Fc-fragment**  
The colour scheme is in agreement to figure 8 and residues important for complex formation are shown in ball-and-stick.

**Fig. 10a:**

30     In the upper part of figure 10a a structure based sequence alignment of the Fc-Receptor ecto-domains is shown. Conserved residues are shaded yellow and identical residues orange. The lower part of the figure shows a part of

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the alignment of human antibody sequences. Residues of the human Fc $\gamma$ RIII in contact with the Fc-fragment in the complex crystal structure are connected by lines (black for hydrophobic interaction, red for salt bridges and blue for hydrogenbridges). Residues from the Fc-receptor in contact with the A-chain of the Fc-fragment are connected with dashed lines and those in contact with the B-chain of the Fc-fragment with solid lines. Red, blue and black lines represent charged, polar and other contacts, respectively.

10 **Fig. 10b:**

In the upper part of figure 10b a structure based sequence alignment of the Fc-Receptor ecto-domains is shown. Conserved residues are shaded yellow and identical residues orange. Conserved residues within the less related Kir and FcA-Receptor sequences are shaded blue. The lower part of the figure shows a part of the alignment of human antibodies with the mouse IgE (mIgE) sequence. Residues of the human Fc $\gamma$ RIII in contact with the Fc-fragment in the complex crystal structure are connected by lines (black for hydrophobic interaction, red for salt bridges and blue for hydrogenbonds). Residues from the Fc-receptor in contact with the A-chain of the Fc-fragment are connected with dashed lines and those in contact with the B-chain of the Fc-fragment with solid lines. Red, blue and black lines represent charged, polar and other contacts, respectively.

25 **Fig. 11 and Fig. 12:**

Fig. 11 and Fig. 12 show an alignment of the produced sFc $\gamma$ R, sFc $\epsilon$ R $\text{Ia}$  and the short form of sFc $\epsilon$ R $\text{II}$  and the produced sFc $\gamma$ R and sFc $\epsilon$ R $\text{Ia}$  without sFc $\epsilon$ R $\text{II}$ , respectively.

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**Table 1: Crystallographic results**

The obtained preliminary crystallographic data are shown in this table.

	Orthorhombic	Tetragonal	Hexagonal
<b>Space group</b>	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub> [19]	P4 <sub>2</sub> 2 <sub>1</sub> 2 [94]	P3 [143]
<b>Unit cell dimensions</b>	a = 40.8 Å, b = 50.9 Å, c = 80.5 Å, α = 90°, β = 90°, γ = 90°	a = 85.7 Å, b = 85.7 Å, c = 63.4 Å, α = 90°, β = 90°, γ = 90°	a = 80.9 Å, b = 80.9 Å, c = 157.0 Å, α = 90°, β = 90°, γ = 90°
R <sub>merge</sub>	5.8%	9.8%	13.6%
<b>Resolution</b>	1.7 Å	2.7 Å	3.8 Å
<b>Unique</b>	18,040	6,616	7,210
<b>Completeness</b>	89.1%	97.1%	63.0%
<b>Multiplicity</b>	3.5	4.4	1.3
V <sub>M</sub> , molecules per asymmetric unit, solvent content	2.09 Å <sup>3</sup> /Da, 1 mol., 41% solvent	2.91 Å/Da, 1 mol, 58% solvent	2.97 Å/Da, 5 mol, 59% solvent

**Table 2: Data collection statistics**

Derivative	Space Group	No. of unique reflections	Multiplicity	Resolution (Å)	Completeness (overall/last shell) (%/%)	R <sub>merge</sub> (%)	No. of sites	Phasing power
NATI	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	18009	3.6	1.74	92.9/86.4	5.5		
NATI	P4 <sub>2</sub> 2 <sub>1</sub> 2	6615	4.5	2.70	97.1/94.3	10.1		
NATI-Baculo	P3 <sub>1</sub> 21	3545	2.5	3.0	93.0/98.9	14.4		
UOAc	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	7722	4.2	2.1	96.8/95.7	7.3	1	1.79
PtPy	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	5520	3.9	2.3	89.7/49.6	10.5	1	1.39

$$R_m = \sum |I_h - \langle I_h \rangle| / \sum \langle I_h \rangle$$

Phasing power:  $\langle F_H \rangle / E$ , where  $\langle F_H \rangle = \sqrt{\sum (F_H^2/n)^{1/2}}$  is the r.m.s. heavy atom structure amplitude.

$E = \sqrt{\sum [(F_{PHC} - F_{PH})^2/n]^{1/2}}$  is the residual lack of closure error with  $F_{PH}$  being the structure factor amplitude and  $F_{PHC} = |F_p + F_h|$  the calculated structure factor amplitude of the derivative.

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**Table 3: Refinement statistics**

Resolution range (Å)	8.0 - 1.74 Å
No. of unique reflections (F>0σ(F))	16252
R factor R <sub>free</sub>	19.4 27.9
No. of atoms per asymmetric unit	
protein	1371
solvent	150
Rms deviation from ideal geometry	
bond length (Å)	0.009
bond angle (°)	2.007
Average B factors (Å <sup>2</sup> )	
protein main chain	18.8
protein side chain	25.2
solvent	36.7
Rms deviation of bonded B factors (Å <sup>2</sup> )	4.1

20 \* R<sub>free</sub>: 5% of the reflections were used as a reference data set and were not included in the refinement.

**Table 4: Residues that contribute to the interdomain contact via side chains**

FcγRIIb	FcγRIIa	FcγRIII	FcγRI	FcεRIa
Asn15	Asn	Ser	Ser	Arg
Asp20	Asp	Asp	Glu	Glu
Gln91	Gln	Gln	Gln	Gln
His108	His	His	His	His
Trp110	Trp	Trp	Trp	Trp

**Table 5: Primers used for the amplification of the FcRs**

Construct	5'-Primer	3'-Primer
sFcγRI	5' - CACCCATATGGCAGTGATCTCTTT - 3'	5' - AGGACTCGAGACTAGACAGGAGTTGGTA AC - 3'

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sFc $\gamma$ RIIa	5' - <b>A</b> CAGTC <b><u>CATATGG</u></b> CAGCTCCCC-3'	5' - <b>A</b> AAAAAA <u>AGCTT</u> CAGGGCACTTGGAC-3'
sFc $\gamma$ RIIb	5' - AATT <u>CCATGGGG</u> ACACCTGCAGCTCCC-3'	5' - CCCA <u>GTCGACAGCCTAA</u> ATGATCCCC-3'
sFc $\gamma$ RIII	5' - <b>A</b> AAAAAA <u>ACATATGG</u> GGACTGAAG-3'	5' - <b>A</b> AAAAA <u>AGCTTAA</u> ACCTTGAGTGATG-3'
sFc $\epsilon$ RIA	5' - GATGCC <u>CATATGG</u> CAGTCCCTCAG-3'	5' - CAAT <u>GGATCCTAAAATTGTAGCCAG</u> -3'
sFc $\epsilon$ RII	5' - <b>A</b> AAAAAA <u>ACATATGG</u> AGTTGCAGG-3'	5' - TGGCT <u>GGATCC</u> CATGCTCAAG-3'

Introduced restriction sites are underlined, start- and stop-codons are depicted as bold-italics

10 Table 6: Refolding Conditions for the FcRs

Construct	Buffer
sFc $\gamma$ RI	0.1M TRIS/HCl, 1.2M arginine, 150mM NaCl, 5mM GSH, 0.5mM GSSG, 0.02% sodium azide, pH 8.0
sFc $\gamma$ RIIa	0.1M TRIS/HCl, 1.4M arginine, 150mM NaCl, 2mM GSH, 0.5mM GSSG, 0.02% sodium azide, pH 8.0
sFc $\gamma$ RIIb	0.1M TRIS/HCl, 1.4M arginine, 150mM NaCl, 5mM GSH, 0.5mM GSSG, 0.02% sodium azide, pH 8.0
sFc $\gamma$ RIII	0.1M TRIS/HCl, 1.0M arginine, 150mM NaCl, 2mM GSH, 0.5mM GSSG, 0.02% sodium azide, pH 8.0
sFc $\epsilon$ RII	0.1M TRIS/HCl, 0.8M arginine, 150mM NaCl, 5mM GSH, 0.5mM GSSG, 0.02% sodium azide, pH 8.3

15 Table 7: Crystallisation Conditions for the FcRs

Construct	Condition	Space group, cell constants	Resolution
sFc $\gamma$ RIIa	26% PEG 8000, 0.2M sodium acetate/acetic acid pH 4.6, 0.02% sodium azide	C2, a=80.4Å, b=49.7Å, c=54.6Å, a=g=90°, b=128.1°	3.0Å

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	sFc $\gamma$ RIIb	33% PEG 2000, 0.2M sodium acetate, 0.02% sodium azide, pH5.4	P212121, a=40.8 $\text{\AA}$ , b=50.9 $\text{\AA}$ , c=80.5 $\text{\AA}$ , a=b=g=90°	1.7 $\text{\AA}$
	sFc $\gamma$ RIII	22% PEG 8000, 0.1M MES/TRIS pH 7.8, 0.02% sodium azide	P22121, a=36.7 $\text{\AA}$ , b=60.3 $\text{\AA}$ , c=85.6 $\text{\AA}$ , a=b=g=90°	2.5 $\text{\AA}$
	sFc $\gamma$ RIII: hFc1	6% PEG 8000, 0.1M MES/TRIS pH 5.6, 0.2M Na/K tartrate, 0.02% sodium azide	P6522, a=b=115.0 $\text{\AA}$ , c=303.3 $\text{\AA}$ , a=b=90°, g=120°	3.3 $\text{\AA}$
5	sFc $\gamma$ RIII	22% PEG 8000, 0.1M MES/TRIS pH 7.8, 0.02% sodium azide	P22121, a=36.7 $\text{\AA}$ , b=60.3 $\text{\AA}$ , c=85.6 $\text{\AA}$ , a=b=g=90°	2.5 $\text{\AA}$

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**Claims**

1. Recombinant soluble Fc receptor characterized by the absence of transmembrane domains, signal peptide and glycosylation.  
5
2. Recombinant Fc receptor according to claim 1, wherein the receptor is a Fc $\gamma$ R or a Fc $\epsilon$ R.
- 10 3. Recombinant Fc receptor according to claim 1 or 2, wherein the receptor is a Fc $\gamma$ RIIb.
4. Recombinant Fc receptor according to any one of claims 1 to 3, wherein the receptor is of human origin.  
15
5. Recombinant Fc receptor according to any one of claims 1 to 4, wherein it contains one of the amino acids as shown in one of SEQ ID NOs:1-6.
- 20 6. Recombinant nucleic acid containing a sequence encoding a recombinant Fc receptor according to any one of claims 1 to 5.
7. Recombinant nucleic acid according to claim 6, wherein it contains one of the sequences as shown in one of SEQ ID  
25 NOs:7-12.
8. Recombinant nucleic acid according to claim 6 or 7, wherein it additionally contains expression control sequences operably linked to the sequence encoding the recombinant Fc receptor.  
30
9. Recombinant nucleic acid according to any one of claims 6 to 8,

wherein it is contained on a prokaryotic expression vector, preferably a pET vector.

10. Host cell characterized by the presence of a recombinant nucleic acid  
5 according to any one of claims 6 to 8.
11. Host cell according to claim 10,  
wherein it is a prokaryotic host cell, preferably an E. coli cell.
- 10 12. Process for the determination of the amount of antibodies of a certain type in the blood, plasma or serum of a patient, characterized by the use of a recombinant soluble Fc receptor according to any one of claims 1 to 5 in an immunoassay and determination of the presence  
of FcR-antibody complexes.  
15
13. Process according to claim 12, wherein the immunoassay is an ELISA and preferably a sandwich assay.
14. Process according to claim 12 or 13, wherein the antibodies to be  
20 determined are IgE antibodies and the recombinant soluble receptor is a Fc $\epsilon$ R.
15. Process according to claim 14 for the determination of a predisposition or manifestation of an allergy.  
25
16. Process according to claim 12 or 13, wherein the antibodies to be determined are IgG antibodies and the recombinant soluble receptor is a Fc $\gamma$ R.
- 30 17. Process for the determination of the immune status of patients with chronic diseases of the immune system, wherein a Fc receptor according to any one of claims 1 to 5 is used in a competitive

immunoassay and the amount of the corresponding sFcRs in the blood, plasma or serum of a patient is determined.

18. Process according to claim 17, wherein the chronic disease is AIDS,  
5 SLE, MM or rheumatoid arthritis.
19. Use of a recombinant soluble Fc receptor according to any one of claims 1 to 5 for the screening of substances in view of their ability to act as inhibitors of the recognition and binding of antibodies to the  
10 respective cellular receptors.
20. Use according to claim 19, wherein recombinant soluble FcγRs are used and recognition and binding of IgG antibodies is of interest.
- 15 21. Pharmaceutical composition containing as active agent a recombinant soluble FcR according to any one of claims 1 to 5.
22. Pharmaceutical composition according to claim 21 for use in the treatment or prevention of autoimmune diseases, allergies or tumor  
20 diseases.
23. Pharmaceutical composition according to claim 21 or 22 for use in the treatment of AIDS, rheumatoid arthritis or multiple myeloma, containing a recombinant soluble FcγR preferably having the amino  
25 acid sequence as shown in SEQ ID NO:1.
24. Crystalline preparation of a soluble recombinant Fc receptor according to claims 1 to 5.
- 30 25. Crystalline preparation of a soluble recombinant Fc receptor / immunoglobulin complex.

26. Use of a crystalline preparation of a recombinant soluble Fc receptor according to any one of claims 1 to 5 for the generation of crystal structure data of Fc receptors.
- 5 27. Use of a crystalline preparation of a soluble recombinant Fc receptor / immunoglobulin complex for the generation of crystal structure data of receptor / Ig complexes and their respective binding sites.
- 10 28. Use of crystal structure data obtained by the use according to claims 26 or 27 for the identification and/or preparation of Fc receptor or immunoglobulin inhibitors.
- 15 29. Use of crystal structure data obtained by the use according to claim 26 or 27 for the identification and preparation of new antibody receptors.
30. Use according to any one of claims 26 to 29 in a computer-aided modelling program.
- 20 31. FcR inhibitor characterized in that it has a three-dimensional structure which is complementary to the recombinant soluble FcR according to any one of claims 1 to 5.
- 25 32. Immunoglobulin-inhibitor, characterized in that it has a three-dimensional structure which is complementary to an Fc receptor binding site of an immunoglobulin.
33. Pharmaceutical composition containing as active agent a FcR inhibitor according to claim 31.
- 30 34. Pharmaceutical composition containing as active agent an immunoglobulin inhibitor according to claim 32.

35. Pharmaceutical composition according to claim 33 or 34 for use in the treatment or prevention of diseases which are due to overreactions or faulty reactions of the immune system.
- 5      36. Pharmaceutical composition according to claim 33, 34 or 35 for the treatment or prevention of allergies, autoimmune diseases or an anaphylactic shock.
- 10     37. Use of a molecule for the modulation of the interaction between Fc receptor and immunoglobulin, characterized in that the molecule is designed or identified using crystal structure data obtained from crystalline preparations according to claims 24 or 25.
- 15     38. Use according to claim 37, wherein the modulation is partial or complete inhibition of binding between Fc receptor and immunoglobulin.
- 20     39. Fc receptor according to claims 1-5, bound to a solid phase.
- 25     40. Fc receptor according to claim 39, wherein the solid phase is a chromatography carrier material.
41. Use of a chromatography carrier material according to claim 40 for the adsorption of immunoglobulins from the blood, plasma or serum of a patient or from culture supernatants of immunoglobulin producing cells.
- 30     42. Use according to claim 41 for the enrichment of antibodies from a patient's blood, serum or plasma or from culture supernatants of immunoglobulin producing cells for the conduction of further tests.

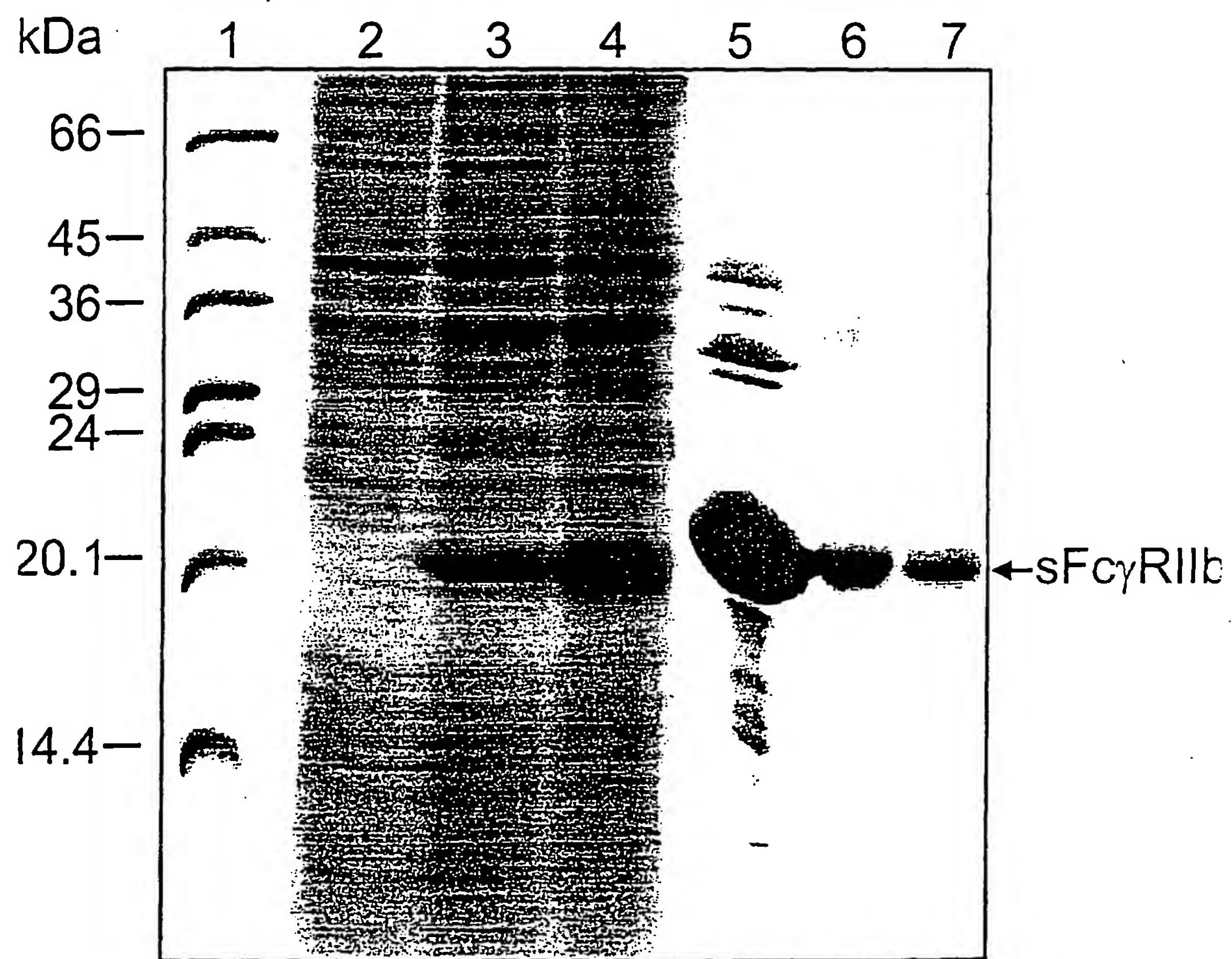


FIG. 1

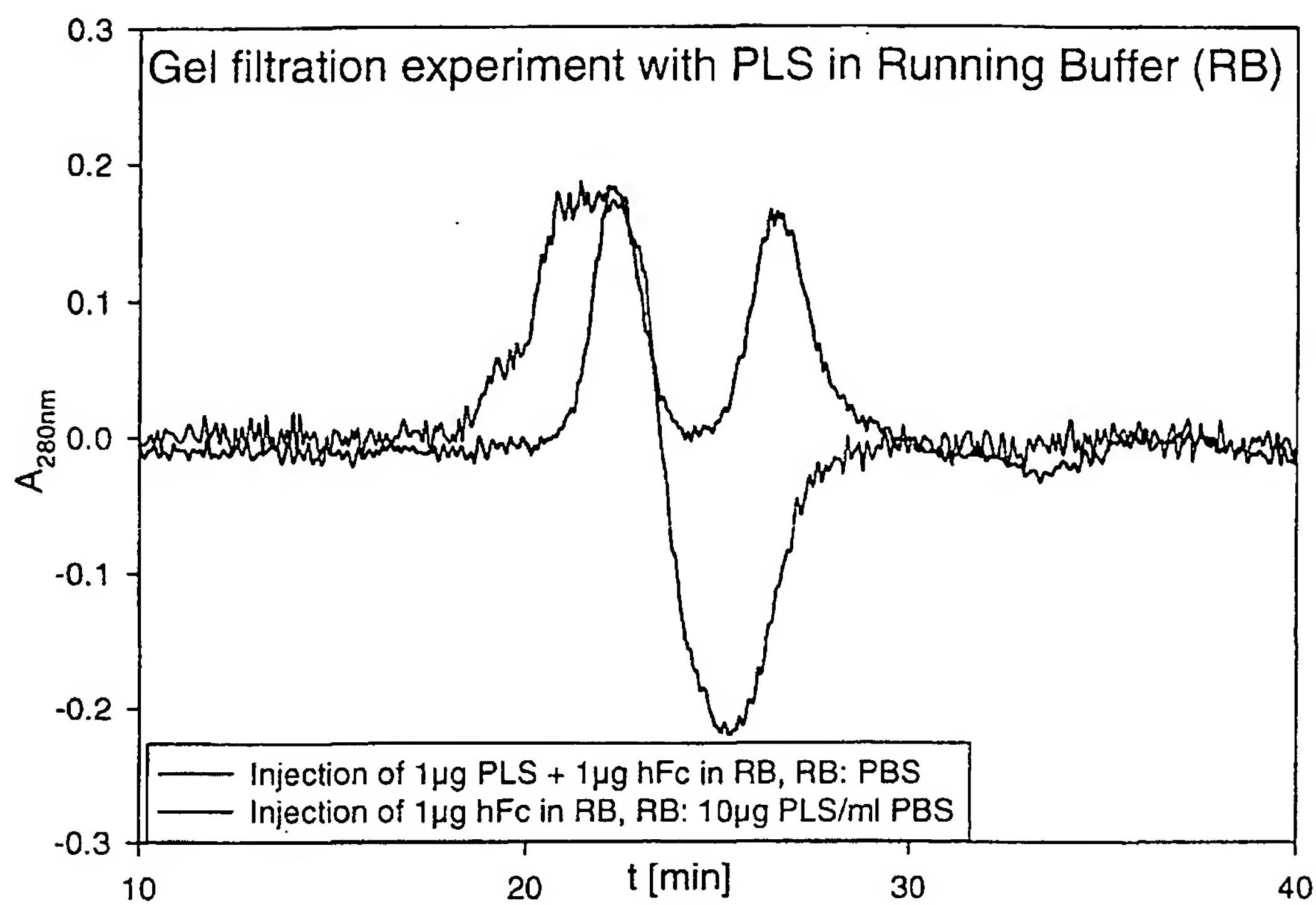
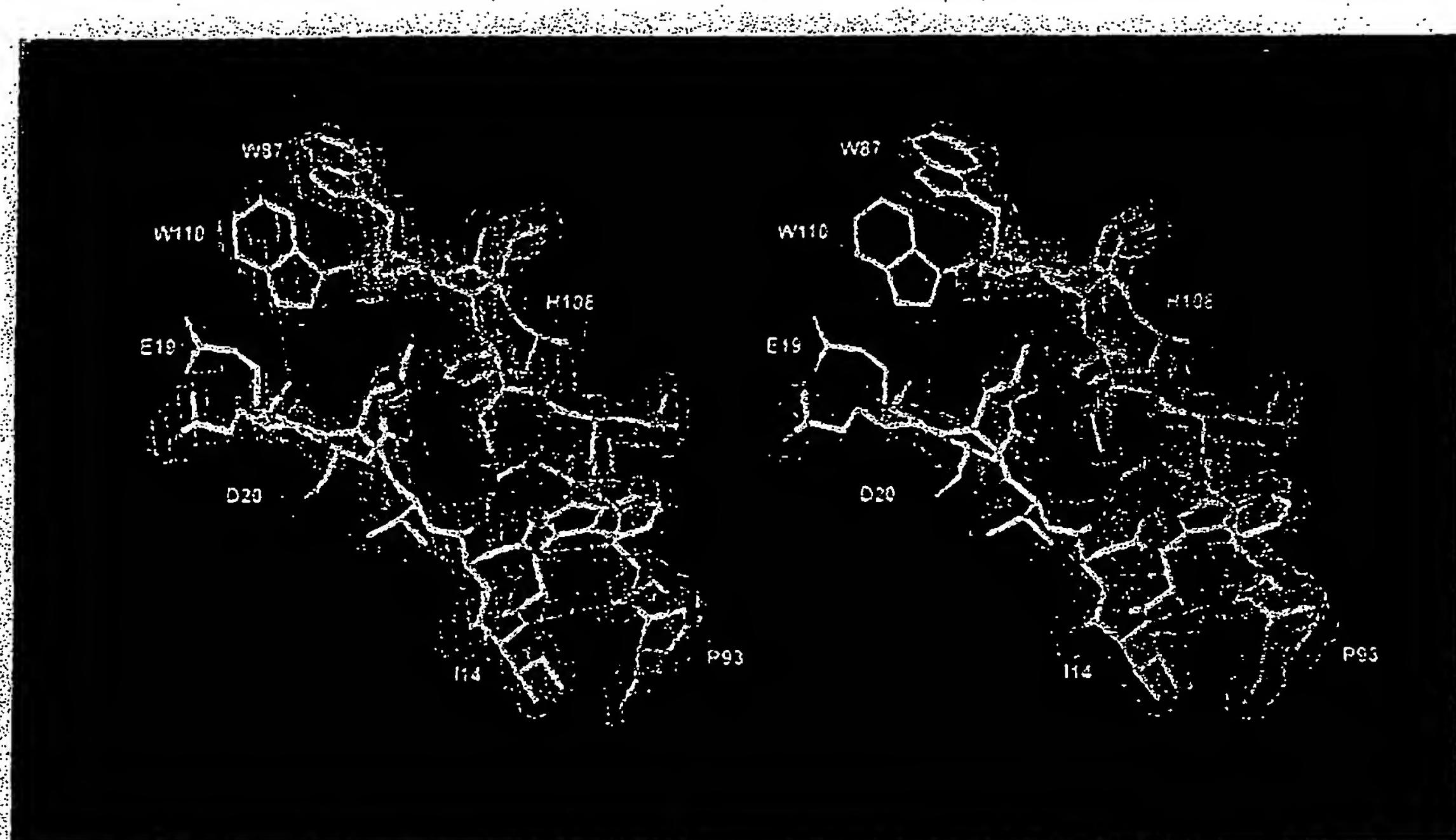


FIG. 2





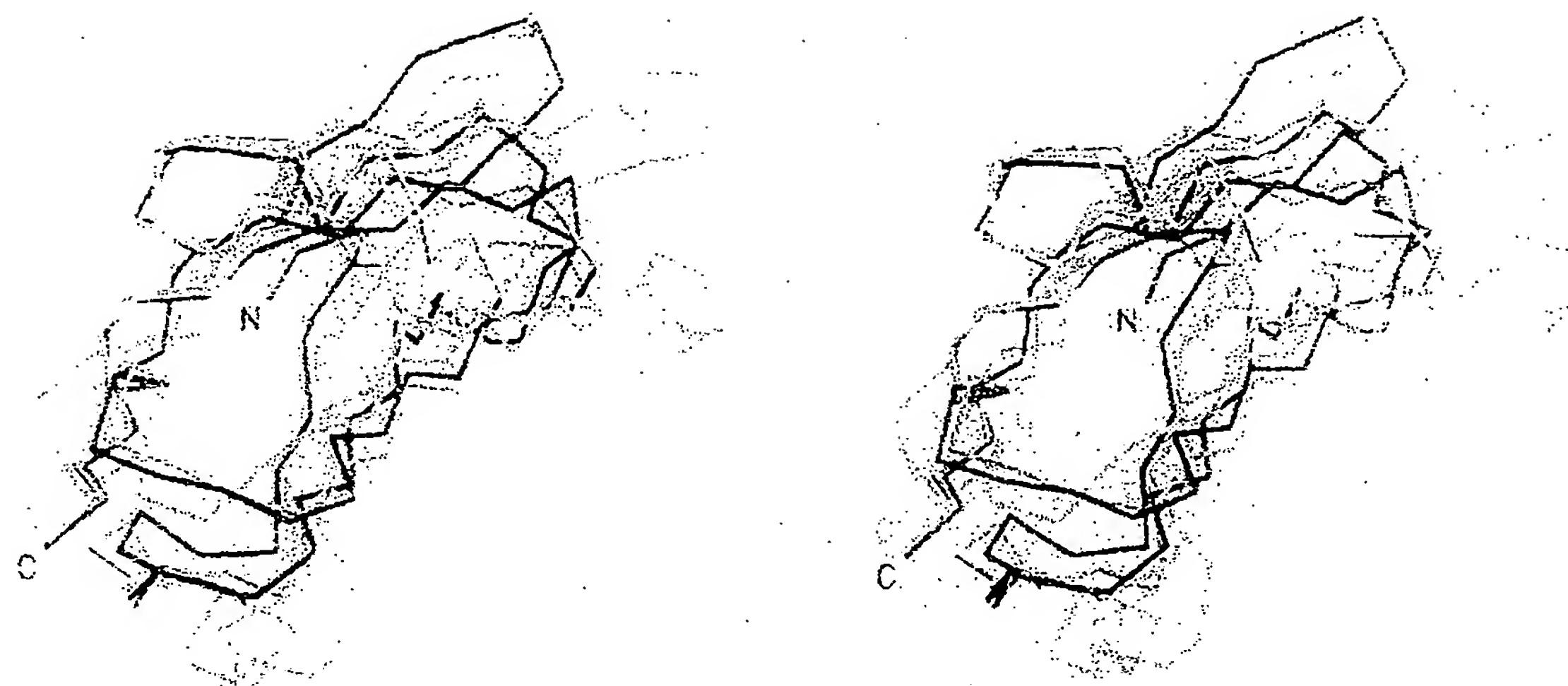
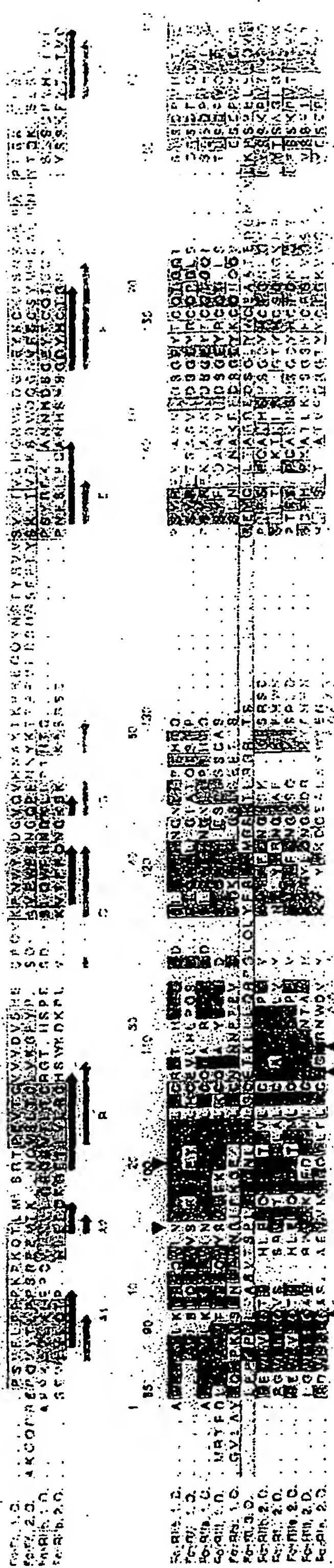
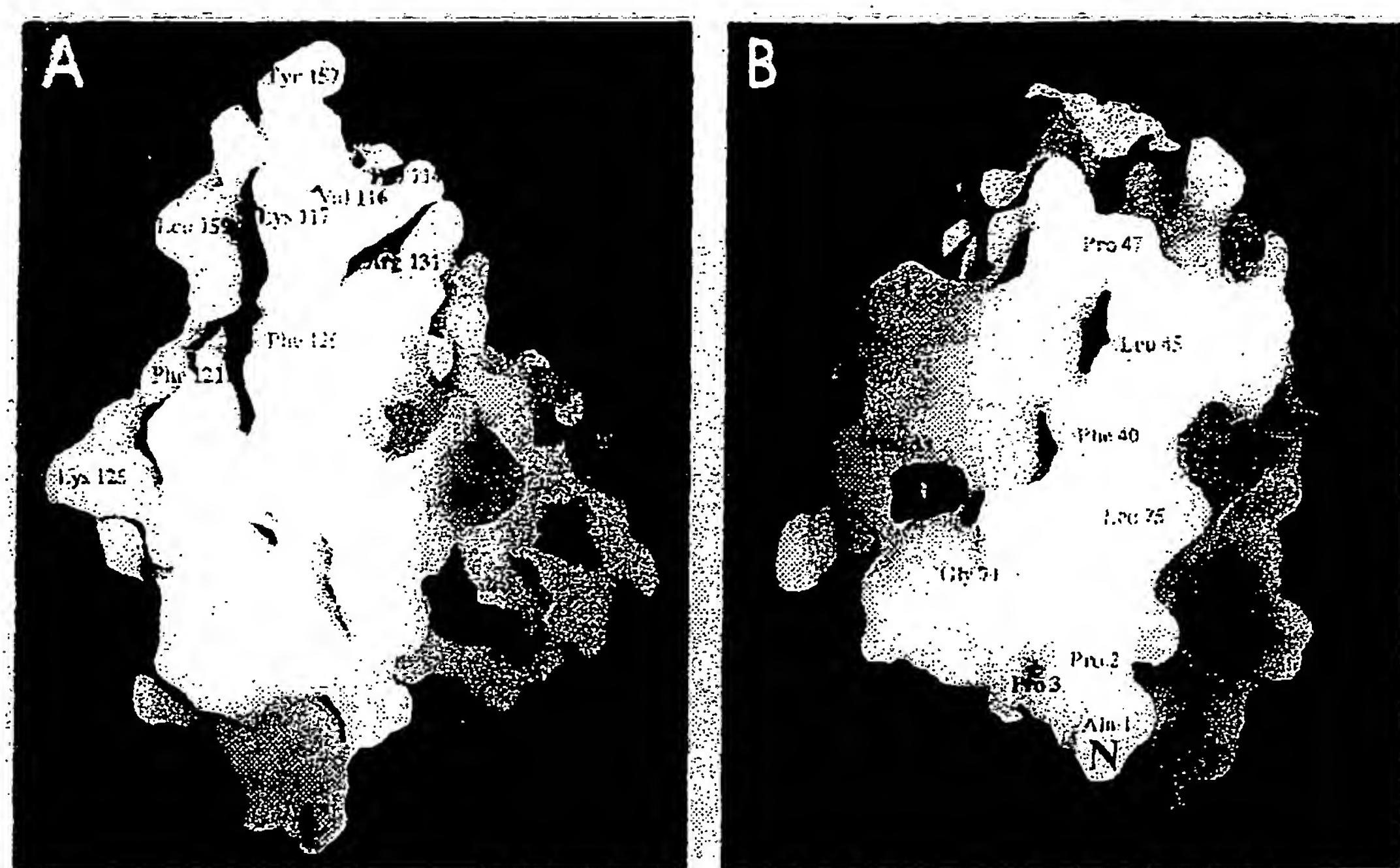


FIG. 5A



TEN. 53



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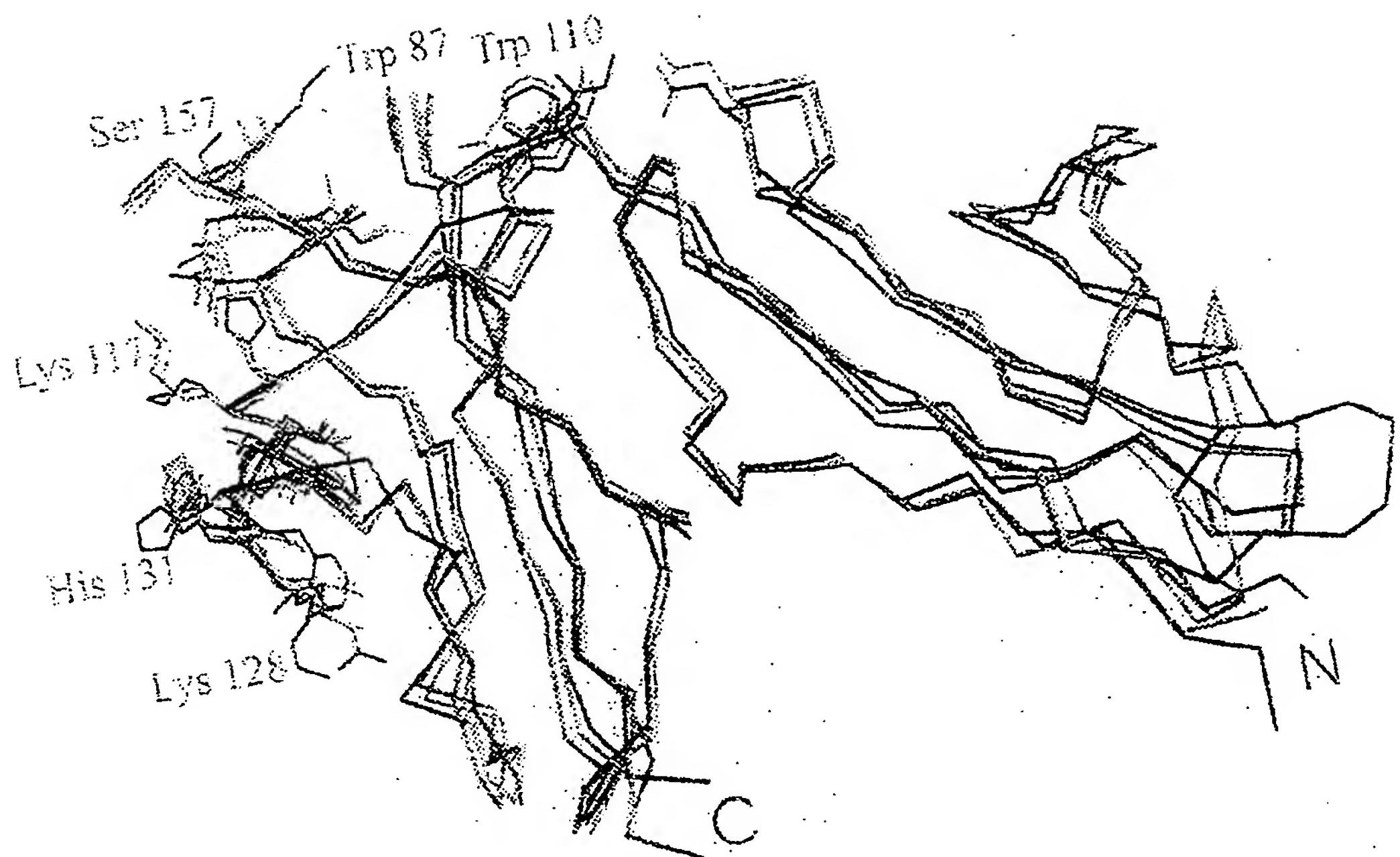


FIG. 7

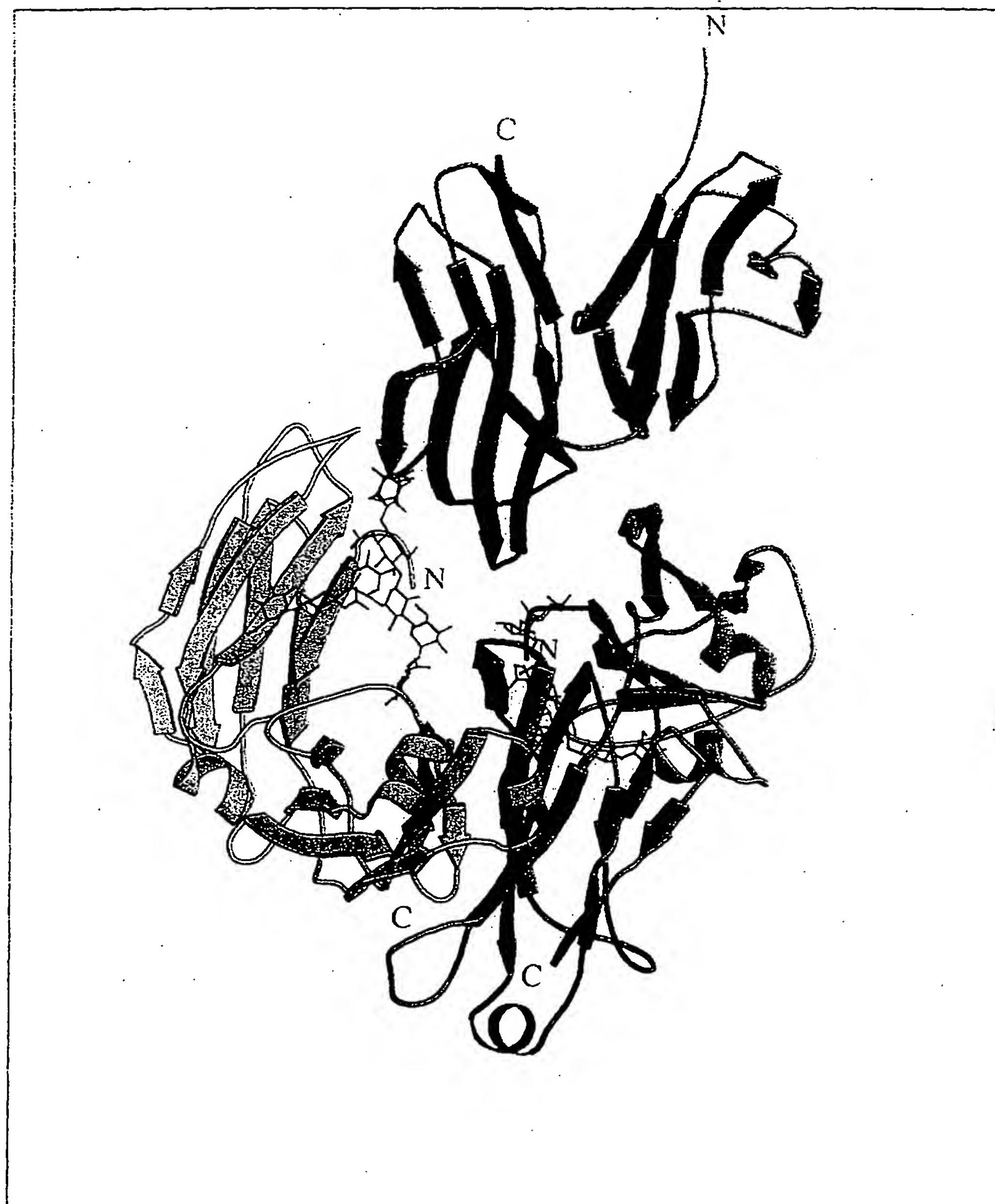


FIG. 8

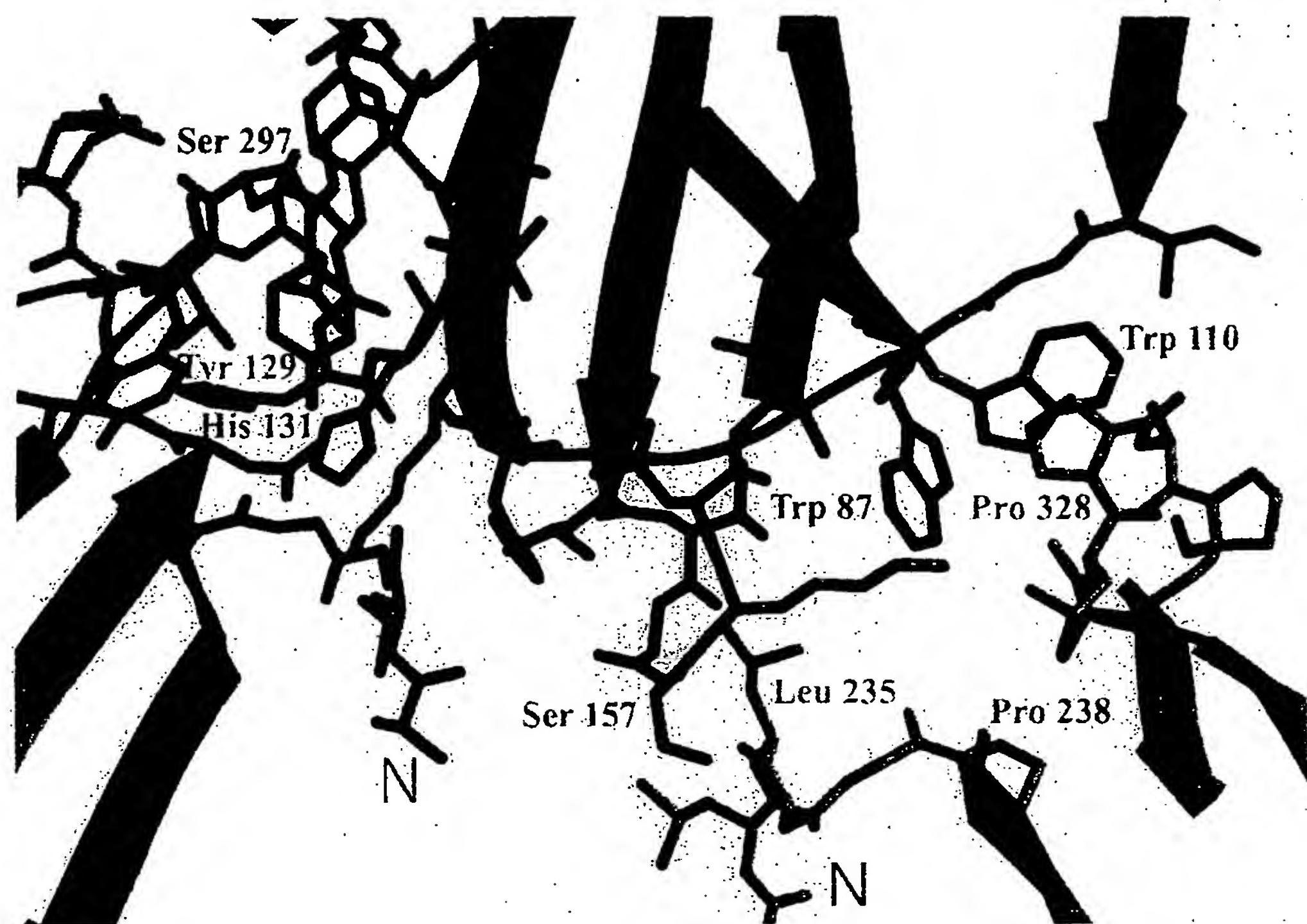


FIG. 9

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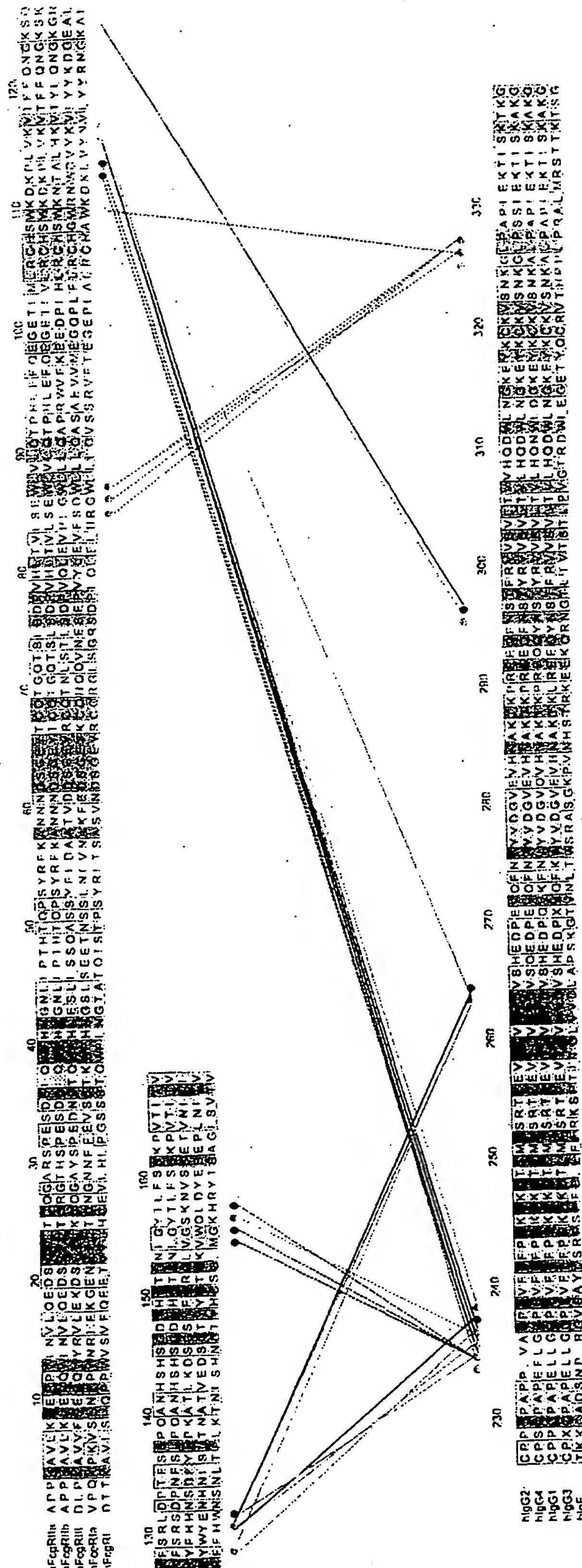
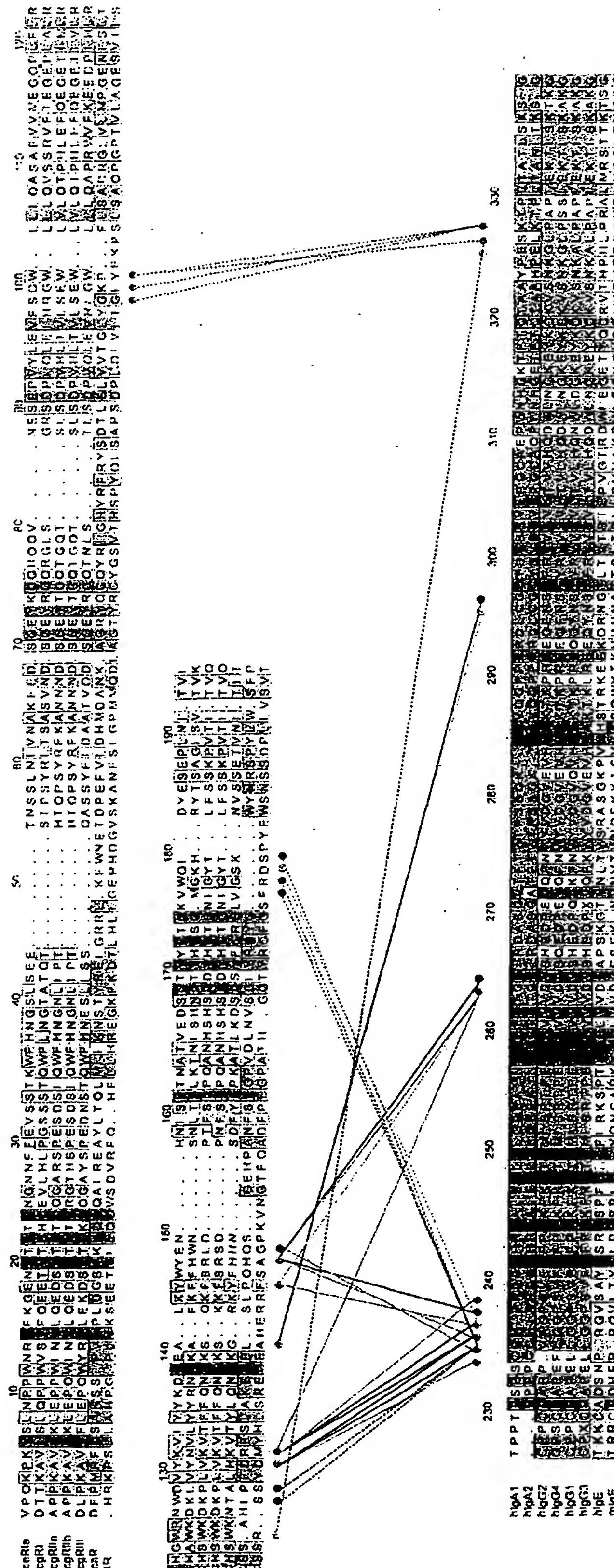


FIG. 10a



Alignment of the produced sFc $\gamma$ R, sFc $\epsilon$ Ria and the short form of sFc $\epsilon$ RII

sFc $\gamma$ RIIa	---MAAPPKAVLKLEPP-WINVQEDSVLTCQGARSPESDSIQWFHN-GNLIPTHTQPS	55
sFc $\gamma$ RIIb	MGTTPAAPPKAVLKLEPQ-WINVQEDSVLTCRGTHSPESDSIQWFHN-GNLIPTHTQPS	58
sFc $\gamma$ RIII	-MRTEDLPKAUVFLEPQ-WYSVLEKDSVTLKCQGAYSPEDNSTQWFHN-ESLISSQASSY	57
sFc $\gamma$ RI	-----MAVISLQPP-WVSVFQEETVTLHCEVLHLPGSSSTQWFLN-GTATQTSTPSY	50
sFc $\epsilon$ Ria	---MAVPQKPKVSLNPP-WNRIFKGENVTLCNGNNFEVSSTKWFHN-GSLSEETNSSL	55
sFc $\epsilon$ RII	-MELQVSSGFVCNTCPEKWINFQRK-----C---YYFGKGTKQWVHARYACDDMEGQLV	50
	* * . . . * .. : *.	
 sFc $\gamma$ RIIa	 YRFKANNNDSG-EYTCQTGQTSLSDPVHLTVLSEWLV-LQTPHLEFQEGETIMLRCHSWK	113
sFc $\gamma$ RIIb	YRFKANNNDSG-EYTCQTGQTSLSDPVHLTVLSEWLV-LQTPHLEFQEGETIVLRCHSWK	116
sFc $\gamma$ RIII	FIDAATVNDSG-EYRCQTNLSTLSDPVQLEVHIGWLL-LQAPRWVFKEEDPIHLRCHSWK	115
sFc $\gamma$ RI	RITSASVNDSG-EYRCQRGLSGRSRSDPIQLEIHRGWLL-LQVSSRVFTEGEPLALRCHAWK	108
sFc $\epsilon$ Ria	NIVNAKFEDSG-EYKCQHQQQNESEPYLEVFSDWLL-LQASAEVVMEGQPLFLRCHGWR	113
sFc $\epsilon$ RII	SIHSPEEQDFLTKHASHTGSWIGLRNLDLKGEFIWVDGSHVDYSNWAPGEPTS-RSQGED	109
	. :* : : .. : * * : .. : .. * ..	
 sFc $\gamma$ RIIa	 DKPLVKVTFQNGK-SQKFSRLDPTFSIPQANHSHSGDYHCTGNIGYTLFSSKPVTITVQ	172
sFc $\gamma$ RIIb	DKPLVKVTFQNGK-SKKFSRSDPNFSIPQANHSHSGDYHCTGNIGYTLSSKPVTITVQ	175
sFc $\gamma$ RIII	NTALHKVTYLQNGK-DRKYFHHNSDFHIPKATLKDSGSYFCRGLVGSKNVSETVNITIT	174
sFc $\gamma$ RI	DKLVNVLYYRNGK-AFKFFHWNSNLTILKTNISHNGTYHCSG-MGKHRYTSAGISVTVK	166
sFc $\epsilon$ Ria	NWDVYKVIIYYKDGE-ALKYWYENHNISITNATVEDSGTYYCTGKVWQLDYESEPLNITVI	172
sFc $\epsilon$ RII	CVMMRGSGRNDAFCDRKLGAWCDRLATCTPPASEGSAESMGPDSRPDPDGRRLPTPSAP	169
	: .. * : .. * .. * .. : ..	
 sFc $\gamma$ RIIa	 VP-----	174
sFc $\gamma$ RIIb	APSSSPMGI-----	185
sFc $\gamma$ RIII	QG-----	176
sFc $\gamma$ RI	ELFPAPVLANASVTSPLLEGNLVTLSCETKLLLQRPGQLQYFSFYMGSKTLRGRNTSSEYQ	226
sFc $\epsilon$ Ria	KAPREKYWLQF-----	183
sFc $\epsilon$ RII	LHS-----	172
 sFc $\gamma$ RIIa	-----	
sFc $\gamma$ RIIb	-----	
sFc $\gamma$ RIII	-----	
sFc $\gamma$ RI	ILTARREDSGLYWCEAATEDGNVLKRSPELELQVLGLQLPTPV	269
sFc $\epsilon$ Ria	-----	
sFc $\epsilon$ RII	-----	

FIG. 11

Alignment the produced sFc $\gamma$ R and sFc $\epsilon$ Ria without sFc $\epsilon$ RII

sFc $\gamma$ RIIa	---MAAPPKAVLKLEPPWINVLQEDSVTLCQGARSPESDSIQWFHNGNLIPTHTQPSYR	57
sFc $\gamma$ RIIb	MGTPAAPPKAVLKLEPQWINVLQEDSVTLCRGTHSPESDSIQWFHNGNLIPTHTQPSYR	60
sFc $\gamma$ RIII	-MRTEDELPKAVVFLEPQWYSVLEKDSVTLKCQGAYSPEDNSTQWFHNESLISSQASSYFI	59
sFc $\gamma$ RI	-----MAVISLQPPWVSVFQEETVTLHCEVLHLPGSSTQWFINGTATQTSTPSYRI	52
sFc $\epsilon$ Ria	---MAVPQPKVSLNPPWNRIFKGENVTLTCNGNNFEVSSTKWFHNGSLSEETNSSLNI	57
	. : *; * * . ; : . * * * . . * : * * * . .	
 sFc $\gamma$ RIIa	FKANNNDSGEYTCQTGQTSLSDPVHLTVLSEWLVLQTPHLEFQEGETIMLRCHSWKDKPL	117
sFc $\gamma$ RIIb	FKANNNDSGEYTCQTGQTSLSDPVHLTVLSEWLVLQTPHLEFQEGETIVLRCHSWKDKPL	120
sFc $\gamma$ RIII	DAATVNDSGEYRCQTNLSTLSDPVQLEVHIGLLLQAPRWVFKEEDIPIHLRCHSWKNTAL	119
sFc $\gamma$ RI	TSASVNDSGEYRCQRGLSGRSRDIQLEIHRGWLLLQVSSRVFTEGEPLALRCHAWKDPLV	112
sFc $\epsilon$ Ria	VNAKFEDSGEYKCQHQVNNESEPYLEVFSDWLLLQASAEVVMEGQPLFLRCHGWRNWDV	117
	*. : * * * * * . : * : * : * * : * . : * : * * * . : :	
 sFc $\gamma$ RIIa	VKVTFFQNGKSQKFSRLDPTFSIPQANHSHSGDYHCTGNIGYTLFSSKPVTITVQVP---	174
sFc $\gamma$ RIIb	VKVTFFQNGKSKKFSRSDPNFSIPQANHSHSGDYHCTGNIGYTLYSSKPVTITVQAPSSS	180
sFc $\gamma$ RIII	HKVTYLQNGKDRKYFHHNSDFHIPKATLKDGSYFCRGLVGSKNVSETVNITITQG---	176
sFc $\gamma$ RI	YNVLYYYRNGKAFKFFHWNSNLTILKTNISHNGTYHCSG-MGKHRYTSAGISVTVKELFPA	171
sFc $\epsilon$ Ria	YKVIYYKDGEALKYWENHNISITNATVEDSGTYYCTGKVWQLDYSEPLNITVIKAPRE	177
	: * : ; ; : * : . : * : . . * . * * : * : * : . : * :	
 sFc $\gamma$ RIIa	-----	
sFc $\gamma$ RIIb	PMGII-----	185
sFc $\gamma$ RIII	-----	
sFc $\gamma$ RI	PVLNASVTSPLLEGNLVTLSCETKLLQRPGLQLYFSFYMGSKTLRGRNTSSEYQILTAR	231
sFc $\epsilon$ Ria	KYWLQF-----	183
 sFc $\gamma$ RIIa	-----	
sFc $\gamma$ RIIb	-----	
sFc $\gamma$ RIII	-----	
sFc $\gamma$ RI	REDSGLYWCEAATEDGNVLKRSPELELQVLGLQLPTPV	269
sFc $\epsilon$ Ria	-----	

FIG. 12

#### Protein sequences of the examples (mutated to yield soluble forms)

>sFc $\gamma$ RI SEQ ID NO: 1  
MAVISLQPPWVSVFQEETVTLHCEVLHLPGSSSTQWFNGTATQTSTPSYRITSASVNDS  
GEYRCQRGLSGRSDPIQLEIHRGWLLLQVSSRVFTEGEPLALRCHAWKDKLVYNVLYRN  
GKAFKFFHWNSNLTILKTNISHNGTYHCSGMGKHRYTSAGISVTVKELFPAPVLNASVTS  
PLLEGNLVTLSCTKLLQRPGLQLYFSFYMGSKTLRGRNTSSEYQILTARREDSGLYWC  
EAATEDGNVLKRSPPELELQLGLQLPTPV

SEQ ID NO: 2

>sFc $\gamma$ RIIa  
MAAPPKAVLKLEPPWINVLQEDSVTLCQGARSPESDSIQWFHNGNLIPTHTQPSYRFKA  
NNNDSGEYTCQTGQTSLSDPVHLTVLSEWLVLQTPHLEFQEGETIMLRCHSWKDKPLVKV  
TFFQONGKSOKFSRLDPTFSIPQANHSHSGDYHCTGNIGYTLFSSKPVTITVOVP

SEQ ID NO: 3

· 3FcγRIIB

KGTPAAPPKAVLKLEPQWINVLQEDSVLTCRGTHSPESDSIQWFHNGNLIPTHTQPSYR  
FKANNNDSGEYTCQTGQTSLSDPVHLTVLSEWLVLQTPHLEFQEGETIVLRCHSWKDPL  
VKVTFFQNGKSKKFSRSDPNFSIPQANHSHSGDYHCTGNIGYTLYSSKPVTITVQAPSSS  
PMGII

SEQ ID NO: 4

>SFcγRIII

MRTEDLPKAVVFL EPQWYSVLEKDSVTLKCQGAYSPEDNSTQWFHNESLISSQASSYFID  
AATVNDSGEYRCQTNLSTLSDPVQLEVHIGWLLQAPRWVFKEEDPIHLRCHSWKNTALH  
KVTYLQNGKDRKYFHHNSDFHIPKATLKDSGSYFCRGLVGSKNVSSETVNITITQG

SEQ ID NO: 5

>SFcERIA

MAVPQKPKVSLNPPWNRIFKGENVLTNCNGNNFFEVSSTKWFHNGSLSEETNSSLIVN  
AKFEDSGEYKCQHQVNNESEPVLLEVFSDWLLLQASAEVVMEGQPLFLRCHGWRNWVDVYK  
VIYYKDGEALKWYENHNISITNATVEDSGTYYCTGKVWQLDYESEPLNITVIKAPREKY  
WTQF

SEQ ID NO: 6

>SFcERII

MDTTQSLKQLEERAARNVSQVSKNLESHHGDQMTQKSQSTQISQELEELRAEQQLRLKSQD  
LELSWNLNGLQADLSSFKSQELNERNEASDLLERLREEVTKLRMELQVSSGFVCNTCPEK  
WINFQRKCYYFGKGTKQWVHARYACDDMEGQLVSIHSPEEQDFLTKHASHTGSWIGLRNL  
DLKGEFIWVDGSHVDYSNWAPGEPTSRSQGEDCVMMRGSGRWNDACDRKLGAWVCDRLA  
TCTPPASEGSAESMGPDSRPDPDGRLPTPSAPLHS

**DNA sequences of the examples (mutated to yield soluble forms)**>sF $\gamma$ RI

SEQ ID NO: 7

1	CATATGGCAG	TGATCTCTT	GCAGCCTCCA	TGGGTCAAGCG	TGTTCCAAGA	GGAAACCGTA	60
61	ACCTTGCACT	GTGAGGTGCT	CCATCTGCC	GGGAGCAGCT	CTACACAGTG	GTTTCTCAAT	120
121	GGCACAGCCA	CTCAGACCTC	GACCCCCAGC	TACAGAACATCA	CCTCTGCCAG	TGTCAATGAC	180
181	AGTGGTGAAT	ACAGGTGCCA	GAGAGGTCTC	TCAGGGCGAA	GTGACCCCAT	ACAGCTGGAA	240
241	ATCCCACAGAG	GCTGGCTACT	ACTGCAGGTC	TCCAGCAGAG	TCTTCACGGA	AGGAGAACCT	300
301	CTGGCCTTGA	GGTGTATGC	GTGGAAGGAT	AAGCTGGTGT	ACAATGTGCT	TTACTATCGA	360
361	AATGGCAAAG	CCTTTAAGTT	TTTCCACTGG	AATTCTAAC	TCACCATTCT	GAAAACCAAC	420
421	ATAAGTCACA	ATGGCACCTA	CCATTGCTCA	GGCATGGGAA	AGCATCGCTA	CACATCAGCA	480
481	GGAATATCTG	TCACTGTGAA	AGAGCTATT	CCAGCTCCAG	TGCTGAATGC	ATCTGTGACA	540
541	TCCCCACTCC	TGGAGGGAA	TCTGGTCACC	CTGAGCTGTG	AAACAAAGTT	GCTCTGCAG	600
601	AGGCCTGGTT	TGCAGCTTTA	CTTCTCCTTC	TACATGGGCA	GCAAGACCC	GCGAGGCAGG	660
661	AACACATCCT	CTGAATACCA	AATACTAACT	GCTAGAAGAG	AAGACTCTGG	GTTATACTGG	720
721	TGGCAGGGCTG	CCACAGAGGA	TGGAAATGTC	CTTAAGCGCA	GCCCTGAGTT	GGAGCTTCAA	780
781	GTGCTTGGCC	TCCAGTTACC	AACTCCTGTC	TAGTCTCGAG			820

>sF $\gamma$ RIIa

SEQ ID NO: 8

1	CATATGGCAG	CTCCCCAAA	GGCTGTGCTG	AAACTTGAGC	CCCCGTGGAT	CAACGTGCTC	60
61	CAGGAGGACT	CTGTGACTCT	GACATGCCAG	GGGGCTCGCA	GCCCTGAGAG	CGACTCCATT	120
121	CAGTGGTTC	ACAATGGGAA	TCTCATTCCC	ACCCACACGC	AGCCCAGCTA	CAGGTTCAAG	180
181	GCCAACAACA	ATGACAGCGG	GGAGTACACG	TGCCAGACTG	GCCAGACCAG	CCTCAGCGAC	240
241	CCTGTGCATC	TGACTGTGCT	TTCCGAATGG	CTGGTGCCTC	AGACCCCTCA	CCTGGAGTT	300
301	CAGGAGGGAG	AAACCATCAT	GCTGAGGTGC	CACAGCTGGA	AGGACAAGCC	TCTGGTCAAG	360
361	GTCACATTCT	TCCAGAATGG	AAAATCCCAG	AAATTCTCCC	GTTTGGATCC	CACCTTCTCC	420
421	ATCCCACAAG	CAAACCACAG	TCACAGTGGT	GATTACCACT	GCACAGGAAA	CATAGGCTAC	480
481	ACGCTGTTCT	CATCCAAGCC	TGTGACCATC	ACTGTCCAAG	TGCCCTGAAG	CTT	533

>sF $\gamma$ RIIb

SEQ ID NO: 9

1	CCATGGGGAC	ACCTGCAGCT	CCCCCAAAGG	CTGTGCTGAA	ACTCGAGCCC	CAGTGGATCA	60
61	ACGTGCTCCA	GGAGGACTCT	GTGACTCTGA	CATGCCGGGG	GACTCACAGC	CCTGAGAGCG	120
121	ACTCCATTCA	GTGGTCCAC	AATGGGAATC	TCATTCCCAC	CCACACGCAG	CCCAGCTACA	180
181	GGTTCAAGGC	CAACAACAAT	GACAGCGGGG	AGTACACGTG	CCAGACTGGC	CAGACCAGCC	240
241	TCAGCGACCC	TGTGCATCTG	ACTGTGCTT	CTGAGTGGCT	GGTGCCTCAG	ACCCCTCACC	300
301	TGGAGTTCCA	GGAGGGAGAA	ACCATCGTGC	TGAGGTGCCA	CAGCTGGAAG	GACAAGCCTC	360
361	TGGTCAAGGT	CACATTCTTC	CAGAATGGAA	AATCCAAGAA	ATTTCGGATCC	TCGGATCCCA	420
421	ACTTCTCCAT	CCCACAAGCA	AACCACAGTC	ACAGTGGTGA	TTACCACTGC	ACAGGAAACA	480
481	TAGGCTACAC	GCTGTACTCA	TCCAAGCCTG	TGACCATCAC	TGTCCAAGCT	CCCAGCTCTT	540
541	CACCGATGGG	GATCATTAG	GCTGTCGAC				569

>sF $\gamma$ RIII

SEQ ID NO: 10

1	CATATGCGGA	CTGAAGATCT	CCCAAAGGCT	GTGGTGTTC	TGGAGCCTCA	ATGGTACAGC	60
61	GTGCTTGAGA	AGGACAGTGT	GACTCTGAAG	TGCCAGGGAG	CCTACTCCCC	TGAGGACAAT	120
121	TCCACACAGT	GGTTTCACAA	TGAGAGCCTC	ATCTCAAGCC	AGGCCTCGAG	CTACTTCATT	180
181	GACGCTGCCA	CAGTCAACGA	CAGTGGAGAG	TACAGGTGCC	AGACAAACCT	CTCCACCCCTC	240
241	AGTGACCCGG	TGCAGCTAGA	AGTCCATATC	GGCTGGCTGT	TGCTCCAGGC	CCCTCGGTGG	300
301	GTGTTCAAGG	AGGAAGACCC	TATTCACCTG	AGGTGTCA	GCTGGAAGAA	CACTGCTCTG	360
361	CATAAGGTCA	CATATTACA	GAATGGAAA	GACAGGAAGT	ATTTCATCA	TAATTCTGAC	420
421	TTCCACATTC	AAAAGCCAC	ACTCAAAGAT	AGCGGCTCCT	ACTTCTGCAG	GGGGCTTGT	480
481	GGGAGTAAAA	ATGTGTCTTC	AGAGACTGTG	AACATCACCA	TCACTCAAGG	TTAAGCTT	538

>sF $\epsilon$ RIa

SEQ ID NO: 11

1	CATATGGCAG	TCCCTCAGAA	ACCTAAGGTC	TCCTTGAACC	CTCCATGGAA	TAGAATATTT	60
61	AAAGGAGAGA	ATGTGACTCT	TACATGTAAT	GGGAACAATT	TCTTGTGAGT	CAGTTCCACC	120
121	AAATGGTTC	ACAATGGCAG	CCTTTCAGAA	GAGACAAATT	CAAGTTTGAA	TATTGTGAAT	180
181	GCCAAATTG	AAGACAGTGG	AGAATACAAA	TGTCAGCACC	AACAAGTTAA	TGAGAGTGAA	240
241	CCTGTGTACC	TGGAAGTCTT	CAGTGA	CTGCTCCTTC	AGGCCTCTGC	TGAGGTGGTG	300
301	ATGGAGGGCC	AGCCCCTCTT	CCTCAGGTGC	CATGGTTGGA	GGAACTGGGA	TGTGTACAAG	360
361	GTGATCTATT	ATAAGGATGG	TGAAGCTCTC	AAGTACTGGT	ATGAGAACCA	CAACATCTCC	420
421	ATTACAAATG	CCACAGTGA	AGACAGTGG	ACCTACTACT	GTACGGCAA	AGTGTGGCAG	480
481	CTGGACTATG	AGTCTGAGCC	CCTCAACATT	ACTGTAATAA	AAGCTCCGCG	TGAGAAGTAC	540
541	TGGCTACAAT	TTTAGGATCC					560

&gt;sFcεRII

SEQ ID NO: 12

1	CATATGGAGT TGCAGGTGTC CAGCGGCTTT GTGTGCAACA CGTGCCCTGA AAAGTGGATC	60
61	AATTCCAAC GGAAGTGCTA CTACTTCGGC AAGGGCACCA AGCAGTGGGT CCACGCCCGG	120
121	TATGCCTGTG ACGACATGGA AGGGCAGCTG GTCAGCATCC ACAGCCCGGA GGAGCAGGAC	180
181	TTCCTGACCA AGCATGCCAG CCACACCGGC TCCTGGATTG GCCTTCGGAA CTTGGACCTG	240
241	AAGGGGGAGT TTATCTGGGT GGATGGGAGC CACGTGGACT ACAGCAACTG GGCTCCAGGG	300
301	GAGCCCACCA GCCGGAGCCA GGGCGAGGAC TGCCTGATGA TGCGGGGCTC CGGTCGCTGG	360
361	AACGACGCCCT TCTGCGACCG TAAGCTGGGC GCCTGGGTGT GCGACCGGCT GGCCACATGC	420
421	ACGCCGCCAG CCAGCGAAGG TTCCCGGGAG TCCATGGGAC CTGATTCAAG ACCAGACCCT	480
481	GACGGCCGCC TGCCCACCCC CTCTGCCCTT CTCCACTCTT GAGCATGGAT CC	532

SEQ ID NO:13

human FcγRIIb2

1	ggctgtgact gctgtgctct gggcgccact cgctccaggg agtgatggga atcctgtcat
61	tttacctgt ccttgccact gagagtgact gggctgactg caagtcccc cagccttggg
121	gtcatatgt tctgtggaca gctgtgctat tcctggctcc tttgtctggg acacctgcag
181	ctcccccaaa ggctgtgctg aaactcgagc cccagtggat caacgtgctc caggaggact
241	ctgtgactct gacatgccgg gggactcaca gccctgagag cgactccatt cagtggttcc
301	acaatggaa ttcattccc acccacacgc agcccagcta caggttcaag gccaacaaca
361	atgacagcgg ggagtacacg tgccagactg gccagaccag cctcagcgac cctgtgcattc
421	tgacagtgt ttctgagtgg ctgggtctcc agaccctca cctggagttc caggagggag
481	aaaccatgt gctgaggtgc cacagctgga aggacaagcc tctggtaag gtcacattct
541	tccagaatgg aaaatccaag aaatttccc gttcgatcc caacttctcc atcccacaag
601	caaaccacag tcacagtggt gattaccatt gcacagggaa cataggctac acgctgtact
661	catccaagcc tgtgaccatc actgtccaag ctcccagctc ttccaccgatg gggatcattg
721	tggctgttgt cactggatt gctgtagctg ccattgtgc tgctgttagtgc ccttgatct
781	actgcaggaa aaagcggatt tcagccaatc ccactaatcc tggatggct gacaaagttg
841	gggctgagaa cacaatcacc tattcacttc tcatgcaccc ggatgctctg gaagagcctg
901	atgaccagaa ccgtatccat tctccattgt cttgcattgg gatttggagaa gaaatcagag
961	aggaaagatc tggtatcc tggctaaat tcccttggg gaggacaggg agatgctgca
1021	gttccaaaag agaaggtttc ttccagagtc atctacctga gtccctgaagc tccctgtct
1081	gaaagccaca gacaatatgg tcccaaatac ccgactgcac cttctgtgt tcagctttc
1141	ttgacatcaa ggctttccg ttccacatcc acacagccaa tccaattaat caaaccactg
1201	ttattaacag ataatacgaa cttggaaat gcttatgtta caggttacgt gagaacaatc
1261	atgtaaatct atatgatttc agaaatgtta aaatagacta acctctacca gcacattaaa
1321	agtgattgtt tctgggtgat aaaattattg atgattttta ttttcttataa ttttctataa
1381	agatcatata ttactttat aataaaacat tataaaaac

## SEQ ID NO:14

human FcεRIα

1 agatctcagc acagtaagca ccaggagtcc atgaagaaga tggctcctgc catggaatcc  
61 cctactctac tgtgtgttagc cttaactgttc ttgcgtccag atggcgtgtt agcagtcct  
121 cagaaaccta aggtctcctt gaaccctcca tggaatagaa tatttaaagg agagaatgtg  
181 actcttacat gtaatggaa caatttctt gaagtcagtt ccaccaaatg gttccacaat  
241 ggcagcctt cagaagagac aaattcaagt ttgaatattg tgaatgccaa atttgaagac  
301 agtggagaat acaaatgtca gcaccaacaa gttaatgaga gtgaacctgt gtacctggaa  
361 gtcttcagtg actggctgct cttcaggcc tctgctgagg tggatgtgaa gggccagccc  
421 ctcttcctca ggtgccatgg ttggaggaac tggatgtgt acaagggtat ctattataag  
481 gatggtaag ctctcaagta ctggtatgag aaccacaaca tctccattac aaatgccaca  
541 gttgaagaca gtggaaaccta ctactgtacg ggcaaagtgt ggcagctgga ctatgagtct  
601 gagccctca acattactgt aataaaagct ccgcgtgaga agtactggct acaattttt  
661 atcccattgt tggatgtat tctgtttgct gtggacacag gattattat ctcaactcag  
721 cagcaggtca catttctctt gaagattaag agaaccagga aaggcttcag acttctgaac  
781 ccacatccta agccaaaccc caaaaacaac tgatataatt aactcaagaa atatttgcaa  
841 cattagttt tttccagcat cagcaattgc tactcaattt tcaaacacag cttgcaatat  
901 acatagaaac gtctgtgctc aaggatttt agaaatgctt cattaaactg agtcaaactg  
961 attaagtggc atgtaatagt aagtgcctaa ttaacattgg ttgaataaat gagagaatga  
1021 atagattcat ttattagcat ttgtaaaaga gatgttcaat ttagatct

## SEQ ID NO:15

human mRNA for high affinity Fc receptor (Fc $\gamma$ RI)

1 gacagatttc actgctccca ccagcttgg aacaacatgt ggttcttgac aactctgctc  
61 ctttgggttc cagtttatgg gcaagtggac accacaaagg cagtatctc tttgcagcct  
121 ccatgggtca gcgtgttcca agagggaaacc gtaaccttgc actgtgaggt gctccatctg  
181 cctgggagca gctctacaca gtggtttctc aatggcacag ccactcagac ctcgaccccc  
241 agctacagaa tcacctctgc cagtgtcaat gacagtggtg aatacaggtg ccagagaggt  
301 ctctcagggc gaagtgaccc catacagctg gaaatccaca gaggctggct actactgcag  
361 gtctccagca gagtcttac ggaaggagaa cctctggct tgaggtgtca tgcgtggaag  
421 gataagctgg tgtacaatgt gctttactat cgaaatggca aagcctttaa gttttccac  
481 tggaaattcta acctcaccat tctgaaaacc aacataagtc acaatggcac ctaccattgc  
541 tcaggcatgg gaaagcatcg ctacacatca gcaggaatat ctgtcactgt gaaagagcta  
601 ttccagctc cagtgtgaa tgcattcttg acatccccac tcctggaggg gaatctggtc  
661 accctgagct gtgaaacaaa gttgcttttg cagaggcctg gtttgcagct ttacttctcc  
721 ttctacatgg gcagcaagac cctgcgaggc aggaacacat cctctgaata ccaaataacta  
781 actgctagaa gagaagactc tgggttatac tggtgcgagg ctgccacaga ggatggaaat  
841 gtccttaagc gcagccctga gttggagctt caagtgttgc gcctccagtt accaactcct  
901 gtctggtttc atgtcctttt ctatctggca gtggaaataa tgtttttagt gaacactgtt  
961 ctctgggtga caatacgtaa agaactgaaa agaaagaaaa agtgggattt agaaatctct  
1021 ttggattctg gtcattgttga gaaggtaact tccagccttc aagaagacag acatttagaa  
1081 gaagagctga aatgtcagga aaaaaagaa gaacagctgc aggaaggggt gcaccggaag  
1141 gagccccagg gggccacgta gcagcggctc agtgggtggc catcgatctg gaccgtcccc  
1201 tgcccacttg ctccccgtga gcactgcgtt caaacatcca aaagttcaac aacaccagaa  
1261 ctgtgtgtct catggtatgt aactcttaaa gcaaataaat gaactgactt caaaaaaaaaa  
1321 a

## SEQ ID NO:16

human Fc $\gamma$ RIIa

1 cccaaatgtc tcagaatgta tgtcccagaa acctgtggct gcttcaacca ttgacagtt  
61 tgctgctgct ggcttctgca gacagtcaag ctgcagctcc cccaaaggct gtgctgaaac  
121 ttgagccccc gtggatcaac gtgctccagg aggactctgt gactctgaca tgccaggggg  
181 ctcgcagccc ttagagcgac tccattca ggttccacaa tggaaatctc attccaccc  
241 acacgcagcc cagctacagg ttcaaggcca acaacaatga cagcggggag tacacgtgcc  
301 agactggcca gaccgcctc agcgaccctg tgcatctgac tgtgcttcc gaatggctgg  
361 tgctccagac ccctcacctg gagttccagg agggagaaac catcatgctg aggtgccaca  
421 gctggaagga caagcctctg gtcaaggta cattttcca gaatggaaaa tcccagaaat  
481 tctcccgaaa ggatcccacc ttctccatcc cacaagcaaa ccacagtcac agtggtgatt  
541 accactgcac aggaaacata ggctacacgc ttttctcatc caagcctgtg accatcactg  
601 tccaaatgtcc cagcatgggc agctttcac caatggggat cattgtggct gtggcatttg  
661 cgactgctgt agcagccatt gttgctgctg tagtggcatt gatctactgc agaaaaaagc  
721 ggatttcage caattccact gatcctgtga aggtgcccattttaggcca cttggacgtc  
781 aaatgattgc catcagaaag agacaactt aagaaaccaa caatgactat gaaacagctg  
841 acggcggcta catgactctg aacccaggg cacctactga cgatgataaa aacatctacc  
901 tgactttcc tcccaacgac catgtcaaca gtaataacta aagagtaacg ttatgccatg  
961 tggtcataact ctcagcttgc tggatggatca aaaaaagagg ggaattgtta aaggaaaaatt  
1021 taaatggaga ctggaaaaat cctgagcaaa caaaaccacc tggcccttag aaatagctt  
1081 aactttgctt aaactacaaa cacaagcaaa acttcacggg gtcataactac atacaagcat  
1141 aagcaaaact taacttggat catttttgtt aaatgcttat gtttagaaata agacaacccc  
1201 agccaatcac aagcagcccta ctaacatata attaggtgac tagggacttt ctaagaagat  
1261 acctacccccc aaaaaacaaat tatgtatattt aaaaaccaacc gattgcctt atttgcttc  
1321 cacattttcc caataaatac ttgcctgtga cattttgcca ctggAACACT aaacttcatt  
1381 aattgcgcct cagattttc ctttaacatc tttttttttt ttgacagagt ctcaatctgt  
1441 tacccaggct ggagtgcagt ggtgctatct tggctcaactg caaacccggcc tcccaaggttt  
1501 aagcgattct tatgcctcag cctcccagta gctgggatta gaggcatgtg ccatcataacc  
1561 cagctaattt ttgtatttttt tttttttttt ttttagtaga gacagggttt cgcaatgttg  
1621 gccaggccga tctcgaactt ctggcctcta gcgtctgccc cgcctcggcc tcccaaaatgt  
1681 ctggatgac cgcattcagcc ccaatgtcca gcctttttaa catcttctt cctatgccct  
1741 ctctgtggat ccctactgct ggtttctgcc ttctccatgc tgagaacaaa atcacctatt  
1801 cactgcttat gcagtcggaa gctccagaag aacaaagagc ccaattacca gaaccacatt  
1861 aagtctccat tttttgcct tgggatttga gaagagaatt agagaggtga ggatctggta  
1921 tttcctggac taaattccct tggggaaagac gaagggatgc tgcagttcca aaagagaagg  
1981 actcttccag agtcatctac ctgagtccta aagctccctg tcctgaaagc cacagacaat  
2041 atggtcccaa atgactgact gcacccctg tgctcagcc gtttttgcata tcaagaatct  
2101 tctgttccac atccacacag ccaatacaat tagtcaaacc actgttattt acagatgttag  
2161 caacatgaga aacgctttag ttacaggtta catgagagca atcatgttaag tctatatgac  
2221 ttcagaaatg taaaataga ctaacctcta acaacaaatt aaaagtgatt gtttcaaggt  
2281 gatgcaatta ttgtatgaccc ttttttttt tctataatga tcatatatta cttttgtat  
2341 aaaacattat aaccaaaac

## SEQ ID NO:17

human Fc $\gamma$ RIII

1       tctttggta cttgtccact ccagtgtggc atcatgtggc agctgctcct cccaaactgct  
61      ctgctacttc tagtttcagc tggcatgcgg actgaagatc tcccaaaggc tgtggtgttc  
121     ctggagcctc aatggtacag cgtgctttag aaggacagtg tgactctgaa gtgccaggaa  
181     gcctactccc ctgaggacaa ttccacacag tggtttcaca atgagagcct catctcaagc  
241     caggcctcgta gctacttcatt tgacgctgcc acagtcaacg acagtggaga gtacaggtgc  
301     cagacaaacc tctccaccct cagtgaccgg gtgcagctag aagtccatat cggctggctg  
361     ttgctccagg cccctcggtg ggtgttcaag gaggaagacc ctattcacct gaggtgtcac  
421     agctggaaga acactgctct gcataaggc acatatttac agaatggcaa agacaggaag  
481     tattttcattc ataattctga cttccacatt caaaaagcca cactcaaaga tagcggctcc  
541     tacttctgca gggggcttgt tgggagtaaa aatgtgtctt cagagactgt gaacatcacc  
601     atcactcaag gtttggcagt gtcaaccatc tcatttcattt ctccacctgg gtaccaagtc  
661     tctttctgct tggtgatggc actcctttt gcagtggaca caggactata tttctctgtg  
721     aagacaaaca tttgaagctc aacaagagac tggaggacc ataaaacttaa atggagaaag  
781     gaccctcaag acaaattgacc cccatccat gggagtaata agagcagtgg cagcagcatc  
841     tctgaacatt tctctggatt tgcaacccca tcatttcag gcctctc

SEQ ID NO: 18

**human Fc&RII**

1 ctcctgctta aacctctgtc tctgacggtc cctgccaatc gctctggtcg  
accccaacac  
61 actaggagga cagacacagg ctccaaactc cactaagtga ccagagctgt gattgtgcc  
121 gctgagtggaa ctgcgttgtc agggagttag tgctccatca tcgggagaat ccaagcagga  
181 ccgccatggaa ggaagggtcaa tattcagaga tcgaggagct tcccaggagg cggtgttgc  
241 ggcgtgggac tcagatcggt ctgctgggac tggtgaccgc cgctctgtgg gctgggctgc  
301 tgactctgtct tctcctgtgg cactgggaca ccacacagag tctaaaacag ctggaagaga  
361 gggctgccccg gaacgtctct caagttcca agaacttggaa aagccaccac ggtgaccaga  
421 tggcgcagaa atccccagtcc acgcagattt cacaggaact ggaggaactt cgagctgaac  
481 agcagagatt gaaatctcag gacttggagc tgtcctggaa cctgaacggg cttcaagcag  
541 atctgagcag cttcaagtcc caggaatttga acgagaggaa cgaagcttca gatttgcgtgg  
601 aaagactccg ggaggagggtg acaaagctaa ggatggagtt gcaggtgtcc agcggctttg  
661 tgtgcaaacac gtgccctgaa aagtggatca atttccaaacg gaagtgcgtac tacttcggca  
721 agggcaccaa gcagtgggtc cacgcccgtt atgcctgtga cgacatggaa gggcagctgg  
781 tcagcatcca cagcccgag gagcaggact tcctgaccaa gcatgccagc cacaccggct  
841 cctggattgg cttcggaaac ttggacctga agggagagtt tatctgggtg gatgggagcc  
901 atgtggacta cagcaactgg gctccagggg agcccaccag ccggagccag ggcgaggact  
961 gcgtgatgat gcggggctcc ggtcgctgaa acgacgcctt ctgcgaccgt aagctggcg  
1021 cctgggtgtg cgaccggctg gccacatgca cgccgcccagc cagcgaaggt tccgcggagt  
1081 ccatgggacc tgattcaaga ccagaccctg acggccgcct gcccacccccc tctgcccctc  
1141 tccactttg agcatggata cagccaggcc cagagcaaga ccctgaagac ccccaaccac  
1201 ggcctaaaag cctctttgtg gctgaaaggt ccctgtgaca ttttctgcca cccaaacgg  
1261 ggcagctgac acatctcccg ctccctctatg gcccctgcct tcccaggagt acaccccaac  
1321 agcacccttcc cagatggaa gtgcccccaa cagcaccctc tccagatgag agtacaccc  
1381 aacagcaccc tctccagatg cagcccccatt tcctcagcac cccaggaccc ggttatcccc  
1441 agctcaggtg gtgagtcctc ctgtccagcc tgcataata aaatggggca gtgatggcct  
1501 ccc

## INTERNATIONAL SEARCH REPORT

Int. Application No.

PCT/EP 99/09440

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C07K14/705 C12N1/21 C12N15/70 G01N33/53  
 G01N33/68 A61K38/17 C07K17/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K C12N G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR 2 739 560 A (ROUSSEL UCLAF) 11 April 1997 (1997-04-11)	1-23
Y	page 3, last line -page 6, line 28	24, 26, 28-30
X	EP 0 614 978 A (ROUSSEL-UCLAF) 14 September 1994 (1994-09-14)	1, 2, 4-13, 16, 21-23
Y	page 3, line 1 -page 7, line 33; examples ---	24, 26, 28-30
		-/-

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

## \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&\* document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

26 April 2000

12/05/2000

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Montero Lopez, B

## INTERNATIONAL SEARCH REPORT

Int. Application No

PCT/EP 99/09440

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 623 053 A (LOUIS N. GASTINEL ET AL.) 22 April 1997 (1997-04-22)	1,4,6, 8-13, 39-42 5,7,24, 26,28-30
Y	column 5, line 60 -column 8, line 67; examples	
X	WO 96 40199 A (UNIVERSITY OF PENNSYLVANIA) 19 December 1996 (1996-12-19)	1,2,4,6, 8-16, 19-22 5,7,24, 26,28-30
Y	page 22, line 24 -page 24, line 14; claims 13-16,36-39; examples I,II	
X	WO 95 09002 A (UNIVERSITY OF PENNSYLVANIA) 6 April 1995 (1995-04-06)	1,2,4,6, 8-13,16, 21,39,40 5,7,24, 26,28-30
Y	page 15, line 4 -page 16, line 22; claims 28-31; examples I,II	
X	EP 0 791 653 A (SCHERING BIOTECH CORPORATION) 27 August 1997 (1997-08-27)	1,2,4,6, 8-11,21, 22 5,7,24, 26,28-30
Y	column 5, line 56 -column 10, line 1; example III	
X	JÉRÔME GALON ET AL.: "Ligands and biological activities of soluble Fcgamma receptors" IMMUNOLOGY LETTERS, vol. 44, January 1995 (1995-01), pages 175-181, XP000574018	1-4,6, 8-13,15, 16,19-22 5,7,24, 26,28-30
Y	page 175, right-hand column, last paragraph -page 177, left-hand column, paragraph 1 page 179, right-hand column, paragraph 3 -page 180, right-hand column, paragraph 3	
X	EP 0 321 842 A (KISHIMOTO, TADAMITSU ) 28 June 1989 (1989-06-28)	1,2, 4-19,21, 22 24,26, 28,30
Y	column 2, line 10 -column 9, line 43; figure 4; examples B,,1-3,6,,7	
		-/-

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/09440

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 259 615 A (KISHIMOTO, TADAMITSU) 16 March 1988 (1988-03-16)	1,2, 4-15, 17-19, 21,22 24,26, 28-30
Y	page 2, line 1 -page 37, line 13	
Y	JANET M. ALLEN ET AL.: "Isolation and expression of functional high-affinity Fc receptor complementary DNAs" JOURNAL OF CRYSTAL GROWTH, vol. 93, no. 1-4, November 1988 (1988-11) - December 1988 (1988-12), pages 378-381, XP000002750 Amsterdam, NL abstract; figure 4	5,7
Y	WILHELM P. BURMEISTER ET AL.: "Crystal structure at 2.2A resolution of the MHC-related neonatal Fc receptor" NATURE, vol. 372, no. 6504, 24 November 1994 (1994-11-24), pages 336-343, XP002135452 LONDON GB the whole document	24,26, 28-30
P,X	WO 99 05271 A (GOULD, HANNAH ET AL.) 4 February 1999 (1999-02-04)	1,2,4,6, 8-15,21, 22,39
	page 4, line 6 - line 19 page 5, line 22 -page 6, line 2 page 7, line 22 -page 8, line 23 page 10, line 31 -page 11, line 31	

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 99/09440

### Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.: 31-38 because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
See FURTHER INFORMATION sheet PCT/ISA/210
  
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

#### Remark on Protest

- The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP 99 09440

### FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 31-38

Present claims 31-38 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is not to be found, however, for any specific example of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, no search has been carried out for claims 31-38.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

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